Dietary and genetic influences on fatty acid composition of sheepmeat

Thesis

DIETARY AND GENETIC INFLUENCES ON FATTY ACID COMPOSITION OF SHEEPMEAT

BY

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UNIVERSITY OF ABERDEEN

THESIS SUBMITTED TO THE OPEN UNIVERSITY FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY

JANUARY 1999

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ABSTRACT

Three experiments were carried out to investigate dietary and genetic factors influencing the *n*-3 polyunsaturated fatty acid content of sheepmeat. In the first experiment, four isoenergetic and iso-nitrogenous diets, based on dried grass with similar fat levels (60 g/kg DM) from different sources; Megalac (control), linseed (α-linolenic acid, C18:3*n*-3), fish oil (eicosapentaenoic, C20:5*n*-3 and docosahexaenoic, C22:6*n*-3 acids) and linseed plus fish oil were fed to four rumen and duodenal cannulated sheep in a 4x4 Latin square design. Biohydrogenation of C18:3*n*-3 was, 80-92 % in all diets, while that of C20:5*n*-3 and C22:6*n*-3 was 58-74 %. Dietary *n*-3 PUFA decreased microbial efficiency ($p<0.05$) measured using the total purine technique as a microbial marker, whilst fish oil depressed fibre digestion in the rumen ($p<0.01$). In experiment two, the same four diets were randomly allocated to 72 lambs from three breeds; Suffolk, Soay and Friesland blocked on live weight. Muscle fatty acid content was 2.4-2.8 % of tissue weight. Compared to the control diet, linseed doubled the quantity of C18:3*n*-3 in muscle and increased levels of C20:5*n*-3 and C22:6*n*-3 by 0.5 ($p<0.001$). Fish oil alone increased C20:5*n*-3 and C22:6*n*-3 by 3-4 times ($p<0.001$), whilst the mixed diet resulted in *n*-3 PUFA levels intermediate between the linseed and fish oil diets. Soay lambs deposited more PUFA on the control diet than Suffolk or Friesland lambs ($p<0.05$). In the third experiment three iso-energetic and iso-nitrogenous diets, with different fat sources, Megalac or formaldehyde treated linseed (protected) with or without fish oil, and with two levels of vitamin E 100 (low) and 500 (high) mg/kg DM were randomly allocated to 96 lambs from two breeds; Suffolk and Scottish Blackface blocked on live weight. Muscle fatty acid content was 2.3-3.1 % of tissue weight but was lower in control fed lambs ($p<0.001$). Compared to the control fat, linseed doubled the quantity of C18:3*n*-3 in muscle and increased levels of C20:5*n*-3 ($p<0.001$), whilst the mixed diet gave intermediate levels. Adipose tissue fatty acid content was 70.0 ± 2 % of tissue weight, but was lower in lambs offered diets containing protected linseed with fish oil ($p<0.05$). Suffolk lambs deposited more C18:3*n*-3, but lower C20:5*n*-3 than the Scottish Blackface lambs ($p<0.01$). Compared to control or mixed diets, linseed doubled the level of C18:3*n*-3 in the adipose tissue ($p<0.001$) and Scottish Blackface lambs deposited less than Suffolk ($p<0.01$). Levels of PUFA were lower in muscle and adipose tissue of lambs on low vitamin E diets ($p<0.01$). Mean tissue vitamin E concentrations were at deficiency levels (>3 mg/kg muscle) in all lambs fed any of the diets. Overall, dietary modifications were more effective than genetic factors in increasing the *n*-3 PUFA in sheepmeat.

Part of the work in this thesis has appeared previously:

WACHIRA, A. M., SINCLAIR, L. A., WILKINSON, R. G., HALLETT, K., ENSER, M. and WOOD, J. D. (1998). Rumen biohydrogenation of polyunsaturated fatty acids and their effect on microbial efficiency in sheep. *British Society for Animal Science pp 36.*

WACHIRA, A. M., SINCLAIR, L. A., WILKINSON, R. G., HEWETT, B., ENSER, M. and WOOD, J. D. (1998). The effects of fat source and breed on fatty acid composition of lamb muscle. *British Society for Animal Science pp.* 38

KURT, E., NUTE, G. R., ENSER, M., RICHARDSON, R. I., WOOD, J. D., WACHIRA, A. M., SINCLAIR, L. A. and WILKINSON, R. G. (1998). Effect of dietary unsaturated fats on fatty acid composition, flavour, oxidative and colour stability of sheepmeat. *44th ICOMST, Barcelona Spain, pp C56*

WACHIRA, A. M., SINCLAIR, L. A., WILKINSON, R. G., DEMIREL, G., ENSER, M. and WOOD, J. D. (1999). The effects of fat source, breed and dietary vitamin E on the fatty acid composition of lamb muscle and adipose tissue. *British Society for Animal Science No. 114.*

ENSER, M., DEMIREL, G., WOOD, J. D., NUTE, G., WACHIRA, A. M., SINCLAIR, L. A. and WILKINSON, R. G. (1999). Impaired deposition of vitamin E in lambs of two breeds fed a dry pelleted complete diet and its effects on meat quality. *British Society for Animal Science No. 40.*

TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

INTRODUCTION

The consumption of fat and in particular saturated fatty acids is considered to be excessive and a major risk factor for CHD, which accounts for 27 % of all deaths in the UK (COMA, Department of Health, 1994). Combined with a sedentary lifestyle, diets providing more than 40 % of dietary energy intake from fat result in positive energy balance with increased incidence of CHD, through increased obesity, blood pressure and blood cholesterol levels (Wiseman, 1997). Saturated fatty acids, especially short and medium chain acids (C12-C16) raise blood cholesterol levels, whilst polyunsaturated fatty acids (PUFA) as a group have a range of positive effects including lowering blood cholesterol. In the past, emphasis on the enhancement of dietary PUFA was through the manipulation of C18 components, in particular linoleic acid (C18:2*n*-6) the precursor for the *n*-6 fatty acids (Budowski, 1989). However, clinical research has demonstrated that *n*-3 long chain PUFA from fish oil (eicosapentaenoic acid, C20:5*n*-3; and docosahexaenoic acid, C22:6*n*-3), whose starting point for synthesis is α-linolenic acid (C18:3*n*-3), are effective in reducing the risk of CHD due to their antithrombogenic nature. Thus current recommendations are that the ratio of polyunsaturated to saturated fatty acids (P:S ratio) in the diet should be increased to a value of 0.45 and intakes of n-3 PUFA should be increased relative to *n*-6 to a value of less than 2.0 (Department of Health 1994). Additionally COMA has recommended that fat should not exceed 35 % of dietary energy, and SFA and *n*-6 PUFA should each contribute no more than 10 % of the dietary energy intake.

More balanced nutritional strategy between the provision of *n*-6 and *n*-3 acids aims to overcome the perceived imbalance in current diets (10:1), compared with that of primitive man (1:1) (British Nutrition Foundation, 1992), which is thought to be involved in several of the so called 'Diseases of Western Civilisation' (James *et al.* 1992). However, modification of current diets to meet health recommendations requires the provision of suitable feeds as well as encouraging people to change their consumption patterns. Furthermore, if the nutritionally desirable food matches the existing products in quality, the change in consumption will be more readily attainable than if a marked alteration in the diet is required. Besides fish meat is one of the major sources of long chain *n*-3 PUFA in the human diet (Gregory *et al.* 1990) and thus has an important role in the provision of these nutrients.

The declining consumption of sheepmeat in the UK has been related to a high price coupled with the high level of fat, especially saturated fatty acids and consequently a low P:S ratio. Set against this low P:S ratio, the *n*-6 to *n*-3 ratio is beneficially low at least when lambs of certain breeds are finished on grass. Enser *et al.* (1996), observed that the mean P:S ratios of beef, lamb and pork muscle at retail was 0.11, 0.15 and 0.58, whilst the *n*-3 to *n*-6 ratios was 2.1, 1.3 and 7.2. A range of C20 and C22 PUFA were also present in the intramuscular lipids of the three species but their concentration in lamb and beef was lower than in pork. Factors controlling the P:S and *n*-6 to *n*-3 ratios of sheepmeat are not well understood. Dietary levels of PUFA are clearly a factor (Christie, 1979), but variable amounts escape biohydrogenation in the rumen. Post absorption variations occur in the extent to which specific PUFA are incorporated into muscle lipids (Scott and Ashes, 1993). Part of the overall variation can be accounted for by dietary levels and part could be genetic (McClelland *et al.* 1976). Enhancing PUFA levels may compromise the oxidative stability and flavour of meat products post-mortem (Pearsons *et al.* 1983). Dietary antioxidants, such as vitamin E, at levels above those required for maintenance has been demonstrated to increase tissue vitamin E levels and consequently enhance shelf life (Wulf *et al.* 1995). However, there are few studies reporting the effects of feeding vitamin E in high PUFA diets in sheep.

The objective of the current study was to investigate whether the P:S ratio in sheepmeat could be increased towards an ideal value of 0.45, and the *n*-6 to *n*-3 ratio to a value of less than 2.0 through dietary and genetic manipulations. Since increases in tissue PUFA decrease oxidative stability, the effects of supra nutritional levels of dietary vitamin E as an antioxidant were also investigated.

CHAPTER 1

LITERATURE REVIEW

1.1 LIPIDS BACKGROUND

1.1.1 Definitions

In chemistry the word "lipid" is used to denote a chemically heterogeneous group of substances having in common the property of insolubility in water, but soluble in non-polar solvents such as chloroform, hydrocarbons or alcohol (Gurr and Harwood, 1996). To the chemist the term lipid embraces a wide range of fatty substances including oils, fats, phospholipids and steroids.

The structural components common to most lipids are the monocarboxylic (COOH), aliphatic fatty acids (Figure 1.1). The fatty acid structure has three important physiological functions. First they are components of biological membranes and the building blocks for phospholipids and glycolipids. Secondly, their derivatives serve as hormones and intracellular second messengers. Thirdly they are fuel molecules, stored as uncharged esters of glycerol (triacylglycerols) which are referred to as neutral lipids (Stryer, 1988).

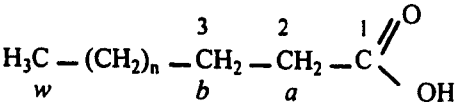


Figure 1.1 Fatty acid carbon atoms

(Adapted from Stryer, 1988)

1.1.2 Nomenclature of fatty acids

The short hand nomenclature consists of two numbers separated by a colon (Gurr and Harwood, 1996). The number before the colon gives the carbon numbers from the carboxyl end with the carboxyl carbon as C1, and the figure after the colon denoting the number of double bonds (Figure 1.1). Thus the symbol C18:0 denotes an 18 carbon fatty acid with no double bonds, whereas C18:2 signifies that there are two double bonds (Table 1.1). The position of the unsaturation is numbered with reference to the first of the pair of carbon atoms between which the double bond occurs. Because of their metabolic connections the double bonds in essential fatty acids are numbered from the methyl end, so that acids derived from

one another can clearly be identified (Figure 1.1). Thus, C18:2 becomes 9,12-C18:2, form the carboxyl end, and 6,9-C18:2 (short hand C18:2*n*-6) from the methyl end.

Unsaturated fatty acids can undergo isomerisation, which may either be positional, or geometrical (Gurr and Harwood, 1996). Positional isomers occur when the double bonds are located at different positions in the carbon chain. For example, a monounsaturated fatty acid C16:1 may have positional isomeric forms with double bonds at C7 and C9, sometimes written as Δ 7 and Δ 9. Geometric isomerism refers to the possibility that the configuration at the double bond can be *cis* or *trans*. In the *cis* form, the two hydrogen substituents are on the same side of the molecule, whilst in the *trans* form they are on opposite sites (Figure 1.2a and b). Thus linoleic acid could be written as *cis* (Δ)9, *cis* (Δ)12 C18:2 or (*cis*, *cis*) 9,12-octadecadienoic acid (Table 1.1) to indicate that it is an 18 carbon fatty acid with *cis* double bonds at carbons 9 and 12 from the carboxyl end (Wahle and James, 1993).

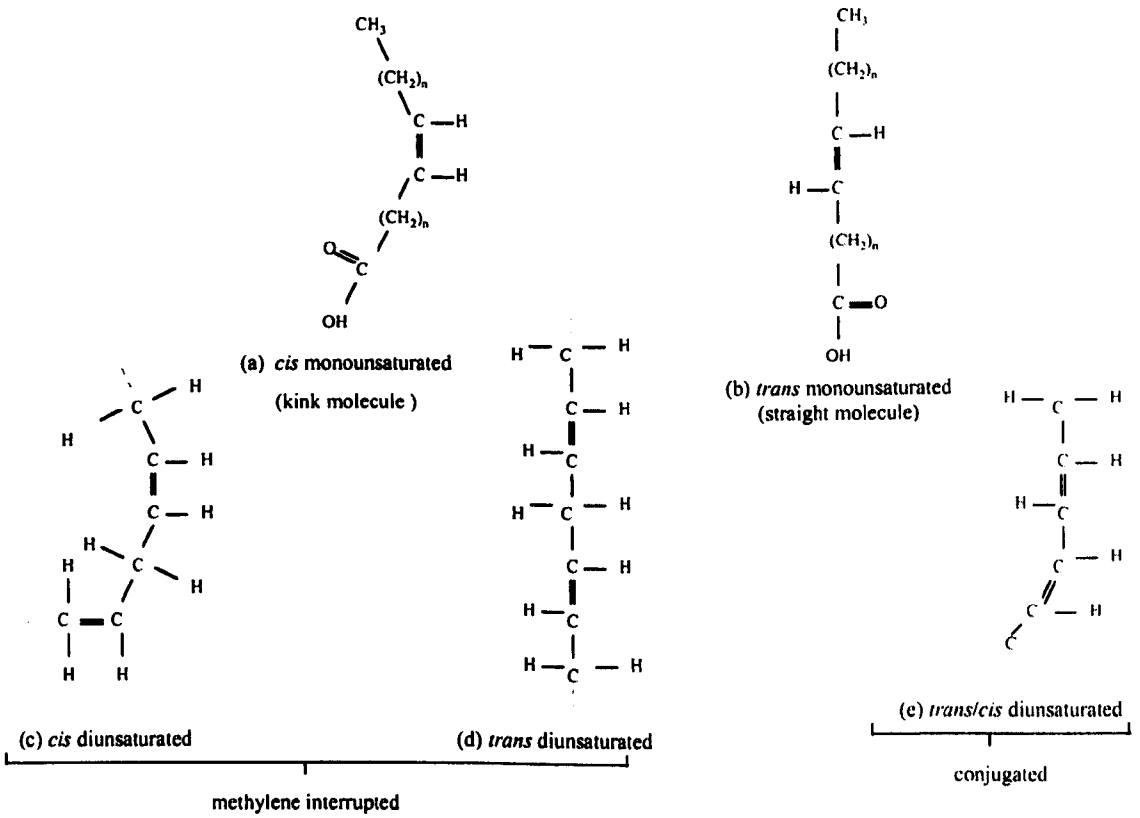


Figure 1.2 Geometric isomerism; Cis/trans configuration of the double bonds

(Adapted from Wahle and James, 1993)

All dienoic acids are derived form monoenoic acids, the position of the second double bond being a function of the biochemical system (Brenner, 1989). Mammals have desaturase which

are capable of removing hydrogen only from the carbon between the existing double bond and the carboxyl group. As such any further desaturation needs to be preceded by chain elongation. Higher plants can carry out desaturation between the existing double bond and the terminal methyl group (Christie, 1983). Double bonds are separated from each other by a methylene grouping (CH_2) (Figure 1.2c and d). However, conjugated fatty acids (no methylene interruption between the double bonds) (Figure 1.2e) have been isolated and are found in lipids from seeds (Tung oil), microorganism and in marine organisms such as sponges (Gurr and Harwood, 1996). The most common conjugated acid is conjugated linoleic acid (CLA) which is present in virtually all foods, but the principal dietary sources are dairy products and other food derived from ruminant products (Chin *et al.* 1992).

Another important feature of biological molecules is their stereochemistry. Lipids based on glycerol have an asymmetry at the central carbon atom of the glycerol molecule (Gurr and Harwood, 1996). Glycerol carbon atoms are thus numbered and the letters *sn* stands for stereochemical numbering (Figure 1.3).

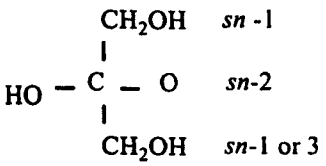


Figure 1.3. Glycerol carbon atoms

(Adapted from Stryer, 1988)

Table.1.1 *Some fatty acids present in animal tissues*

Shorthand name	Systemic name	Common name	Melting point (°C)	Source
C14:0	n-Tetradecanoic	Myristic	54.4	Food or synthesis
C15:0	n-Pentadecanoic	-[2]	52.3	Food or synthesis
C16:0	n-Hexadecanoic	Palmitic	62.9	Food or synthesis
C16:1	*cis*-9-Hexadecanoic	Palmitoleic	0.0	Food or synthesis from palmitic acid
C17:0	n-Heptadecanoic	Margaric	61.3	Food or synthesis
C17:0 br[1]	14-Methyl hexadecanoic	-	39.5	Food or synthesis
C18:0	n-Octadecenoic	Stearic	69.9	Food or synthesis
C18:1	*cis*-9-Octadecenoic	Oleic	13.4	Food or synthesis from stearic acid
C18:1	*trans*-11-Octadecenoic	Vaccenic	39.0	Rumen biohydrogenation
C18:2	*cis*-9,12,-Octadecadienoic	Linoleic	-5.0	Food
C18:3	*cis*-9,12,15-Octadecatrienoic	α-Linoleic	-11.0	Food
C20:0	n-Eicosanoic	Arachidic	75.4	Food or synthesis
C20:4	*cis*-5,8,11,14-Eicosatetraenoic	Arachidonic	-49.5	Food or synthesis from linoleic acid
C20:5	cis-5,8,11,14,17-Eicosapentaenoic	-	-	Synthesis from α-linolenic acid
C22:0	n-Docosanoic	Behenic	80.0	Food or synthesis
C22:1	*cis*-13-Docosenoic	Eurcic	33.5	Food
C22:5	*cis*-7,10,13,16,19-Docosapentaenoic	Clupanodonic	-78.0	Synthesis from α-linolenic acid
C22:6	*cis*-4,7,10,13,16,19-Docosahexaenoic	-	-	Synthesis from α-linolenic acid

br[1] = Branched chain; -[2] = Not known; *Compiled from: Christie (1983); Enser (1984); Gurr and Harwwod (1996)*

1.1.3 Fatty Acids

The common fatty acids of plant and animal origin contain an even number of carbons (4-24) in straight chains with a terminal carboxyl group. These maybe fully saturated or contain one or more double bonds, which are generally but not always of a *cis*-configuration (Gurr and Harwood, 1996). Fatty acids of the animal origin can be subdivided into three families or saturated, monounsaturated (monoenoic) and polyunsaturated fatty acids (PUFA).

1.1.3.1 Saturated fatty acids

Most saturated fatty acids are straight chain structures with an even number of carbon atoms (Table 1.1). Those containing 12-24 carbon atoms are the most common in animal tissues, although shorter chain fatty acids (C4-C10) occur in milk (Enser, 1984). Myristic acid (C14:0) is a minor component of the animal lipids, but a major constituent of seed oil from the family myristicaceae. Palmitic acid (C16:0) is the commonest saturated fatty acid in both animals and plants (Figure 1.4a), while stearic acid (C18:0) is relatively common and may sometimes be more abundant than C16:0 (Christie, 1983). Longer chain fatty acids are less frequently in animal tissues, but can occur in larger amounts in fish and bacterial species. Because of the lack of functional groups other than the carboxyl group saturated fatty acids longer than C10 are comparatively inert chemically and can be subjected to vigorous chemical conditions.

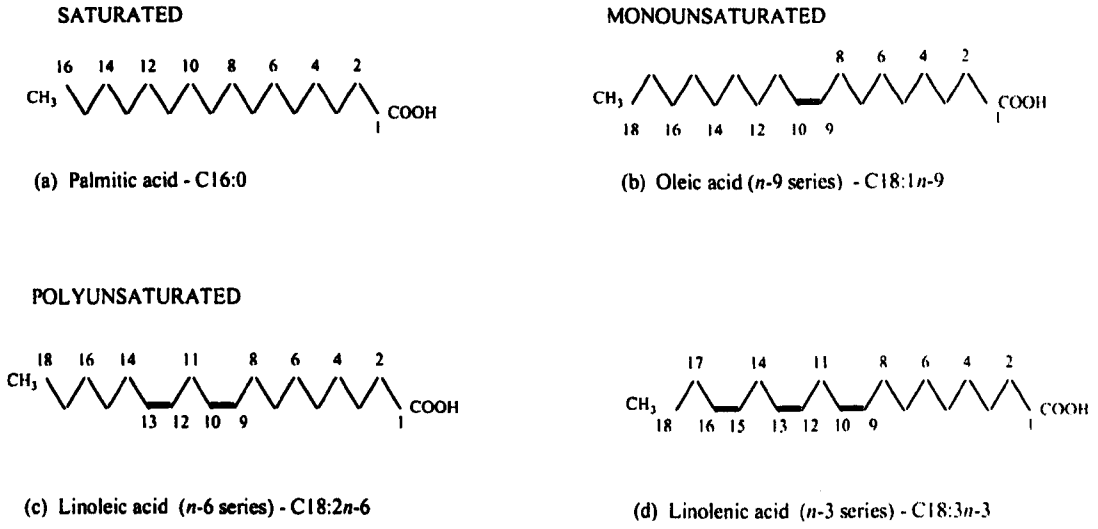


Figure 1.4 Structures of important fatty acids

(Adapted from Gurr, 1994)

1.1.3.2 Monounsaturated fatty acids

These are straight chain even-numbered fatty acids of C16 to C22 containing a double bond of the *cis*-configuration (Gurr and Harwood, 1996). Often the *cis* bond begins at the position 9 (Δ9) although a given fatty acid may have the double bond in a number of different position. Monoenoic acids with double bonds in the *trans*-configuration are rare in nature (Table 1.1). The *cis* configuration introduces a kink into the average molecular shape while the *trans* double bond ensures that the fatty acid has properties similar to those of an equivalent chain length saturated acid (Figure 1.2a and b). Monoenoic fatty acids are more susceptible to chemical attack by oxidising agents than the corresponding saturated acids (Wahle and James, 1993).

Oleic acid (C18:1) is the most abundant monoenoic acid of all and is found in virtually all animals and plant lipids (Figure 1.4b). The *trans* isomer of oleic acid (elaidic acids) is rarely found, but *trans* vaccenic acids, a by-product of rumen biohydrogenation is found in low concentrations in ruminant lipids (Enser, 1984). Many different positional isomers of monoenoic acids may be present in a single natural lipid, for example five different *cis*-octadecenoic acids and eleven different *trans*-octadecenoic have been found in bovine milk triacylglycerides (Christie, 1983). Palmitoleic acid (C16:1) is a component of most animal fats and may be present in greater amounts in fish and some seed oils. Shorter chain acids occur in milk but are rarely found in significant amounts in other tissues. Monoenoic acids of C20 and C22 are minor components of most animal lipids but are found in appreciable amounts in seed oil (e.g. rapeseed oil) and in fish oil. Odd chain monoenoic fatty acids are minor components of animal lipids but may be present in larger amounts in some fish oil and in bacterial lipids (Enser, 1984).

1.1.3.3 Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) of animal and plant lipids can be subdivided into several families or series (*n*-6, *n*-3, *n*-9 and *n*-7), according to their biosynthetic derivation from single specific fatty acid precursors (Brenner, 1989). The acids in each family contain two or more *cis*-double bonds, generally separated by a single methylene group ($-CH_2$) and have the same terminal structure (-COOH) (Figure 1.4c and d). The more the double bond in PUFA, the greater the susceptibility to oxidative attack.

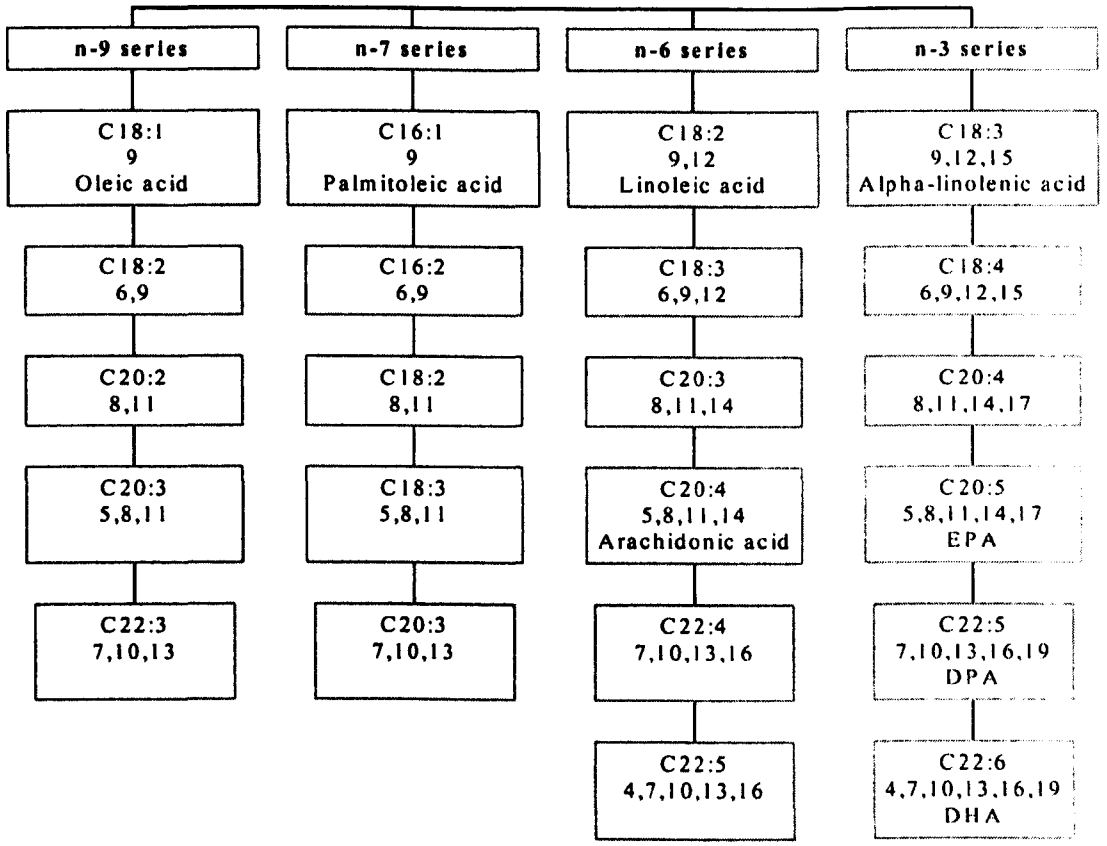


Figure 1.5 Biosynthetic relationships between unsaturated fatty acids

(Adapted from Sprecher and James, 1981; Enser, 1984)

(a) *n*-6 PUFA

Linoleic acids or C18:2*n*-6 (*cis*-9, *cis*-12 octadecadienoic acid, Figure 1.4c) is the commonest and simplest fatty acids found in both plant and animal tissue (Gurr and Harwood, 1996). It is an essential fatty acid (EFA) in animal diets and as such cannot be synthesised by animals, and is a major precursor for a family of other fatty acids, which are produced by desaturation and chain elongation (Figure 1.4). Arachidonic acid or C20:4*n*-6 (*cis*-5, *cis*-8, *cis*-11, *cis*-14 eicosatetraenoic acid) is the most important metabolite of C18:2*n*-6, and a major constituents of the complex lipids of animal tissues but is rarely found in plants (Figure 1.5). In animals C20:4*n*-6 is the major precursor for biologically active C20 compounds known as eicosanoids. Gamma linoleic acid or γ-C18:3*n*-6 (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid) is an important intermediate in the biosynthesis of C20:4*n*-6 from C18:2*n*-6 and occurs in minor amounts in animal tissues but is founds in some seed oils e.g. Linola ™ (Gurr and Harwood, 1996).

(b) *n*-3 PUFA

Alpha linolenic acid or C18:3*n*-3 (*cis*-9, *cis*12, *cis*-15-octadecatrienoic acid, Figure 1.4d) is a major constituent of plant lipids and is rarely found in significant amounts in animal lipids (Christie, 1983). However, it is an essential fatty acid and a precursor for the *n*-3 fatty acids in animal lipids (Figure 1.5). Eicosapentaenoic acid or C20:5*n*-3 (5,8,11,14,17-eicosapentaenoic acid, EPA) and docosahexaenoic acids or C22:6*n*-3 (4,7,10,13,16,19-docosahexaenoic acid, DHA), in particular are found in many animal tissues as major components of the complex lipids (phospholipids), and are found in large amounts in fish oils (Brenner, 1989).

(c) *n*-9 and *n*-7 PUFA

Oleic acid (C18:1*n*-9) can also be the primary precursor of 5,8,11-eicosatrienoic acid a minor component of animal lipid but can assume significance in animals deficient in essential fatty acids (Brenner, 1989). There is in addition a family of PUFA derived from C16:1*n*-7 (Figure 1.5).

1.1.4 Lipid Classes

Lipids can be divided into two broad classes; 'simple' which can be hydrolysed to give one or two types of products per mole and 'complex' which contain three or more hydrolysis products per mole (Christie, 1983).

1.1.4.1 Simple lipids

Simple lipids contain fatty acids esterified to glycerol usually as the alcohol component or a sterol the most abundant of which is cholesterol (Christie, 1983).

(a) Triacylglycerides

Triacylglycerides (TAG) consist of three moles of fatty acids esterified to each hydroxyl group of the trihydric glycerol molecule (Stryer, 1988). TAG are the most abundant single lipids class, constituting up to 95 % of the weight of the adipose tissue in very fat animals (Enser, 1984). Diacylglycerides and monoacylglycerides contain two or one mole of fatty acid per mole of glycerol, respectively and are rarely present in more than trace amounts in animal and plant tissues. However, 1,2 diacyl-*sn*-glyceride is important biosynthetically as a precursor for the TAG and complex lipids (Gurr and Harwood, 1996).

Fatty acids are not esterified at random to the glycerol hydroxyl groups in animals. In most species the *sn*-2 position in the adipose tissue TAG is occupied by an unsaturated fatty acids, but in pigs and human milk it is occupied by a saturated fatty acid, mainly palmitic acid

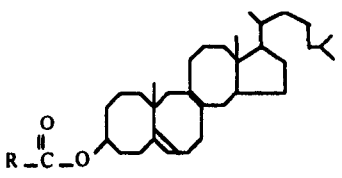
(Enser, 1984). Since the ester bonds of the TAG cannot be distinguished chemically, a stereochemistry of enzymes has been used to determine the fatty acids present in each position (Miller *et al.* 1981). Evidence suggest that fatty acids occupying any one position are independent of the acids esterified to the other three positions (Christie and Moore, 1970 and Miller *et al.* 1981).

(b) Cholesterol and cholesterol esters

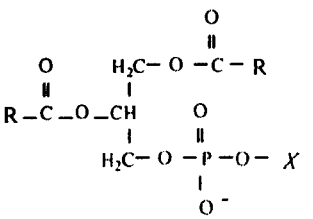
Cholesterol is the commonest member of a group of steroids with a tetracyclic ring system (Christie, 1983, Figure 1.7a). In animal tissues it occurs in the free state in association with esterified lipids in cell membranes and in serum lipoproteins. It is also found esterified to fatty acids (cholesterol esters) in animal tissues, especially in the liver, adrenal glands and plasma. Plant tissue contain related sterols e.g. β-sitosterol, ergosterol and stigmasterol but only trace amounts of cholesterol.

1.1.4.2 Complex lipids

Complex lipids can be subdivided into three classes. 1. Glycerolphospholipids (also known as phosphoglycerides or phosphatidic acid), which consist of glycerol, fatty acids, inorganic phosphate and an organic base (Figure 1.6b). 2. Glycoglycerolipids (also known as glycosyldiglycerides) which consist of glycerol, fatty acid and carbohydrate. 3. Sphingolipid which consists of a long chain base, fatty acid and inorganic phosphate, carbohydrate or other complex organic compounds (Christie, 1983).



(a) Cholesteryl ester



(b) Phosphatidic acid or glycerophospholipid

X	*Name of phospholipid*
Choline	Phosphatidyl choline (lecithin)
Ethanolamine	Phosphatidyl ethanolamine
Serine	Phosphatidyl serine
Myo-inositol	Phosphatidyl inositol

Figure 1.6 Structures of major complex lipids in animal tissues

(Adapted from Enser, 1984)

In glycerophospholipids and glycoglycerolipids the non-fatty acid components are linked almost invariably to position *sn*-3 of the glycerol moiety. The term glycolipid is used to describe any compound containing one or more monosaccharide residues linked by a glycosyl linkage to the lipid, whilst phospholipids are lipid molecules containing phosphoric acid as a mono or diester and so include the glycerophospholipids (Christie, 1983). Some common glycerophospholipids in muscle lipids are;

(a) Glycerolphospholipid (Phosphatidic acid)

Phosphatidic acid or 1,2-diacyl-*sn*-glycerol-3-phosphate (Figure 1.6b) is the precursor for all other glycerophospholipids and of triacylglycerides (Enser, 1984). Glycero-3-*sn*-phosphate is the biosynthetic precursor of phosphatidic acid, although dihydroxyacetone phosphate may be important in some circumstances. In common with the glycerolphospholipids in animal tissues, a saturated or a monoenoic fatty acid occupies position *sn*-1 while position *sn*-2 characteristically contains the PUFA (Figure 1.6b).

(b) Phosphatidylcholine

Phosphatidylcholine (PC) is commonly termed as 'lecithin' and is the most abundant glycerolphospholipid in animal tissues and a major component of plant tissue and microorganism (Christie, 1983). Position *sn*-1 of the PC of animal origin is occupied by a saturated fatty acid while position *sn*-2 may contain the C18, C20 and C22 PUFA or MUFA. Lysophosphatidylcholine (only one of the two available positions of glycerol is esterified to a fatty acid), are often found in small amount in animal tissues when PC is present (Enser, 1984). It is generally believed that position *sn*-1 is esterified but the acyl group migrates and as such both isomers may exist in animal tissue.

(c) Phosphatidylethanolamine

Phosphatidylethanolamine (PE) is the second most abundant class of glycerophospholipid and is present in large quantities in the animal and plant tissue and is frequently the major lipid class in bacteria (Gurr and Harwood, 1996). PE in animal tissue usually contains more PUFA than the PC from the same tissue and the acids are concentrated in position *sn*-2. Lysophosphatidylethanolamine, (only one of the two possible positions of glycerol moeity is esterified) is found frequently in animal tissues (Christie, 1983). Because of the ease with which the acyl group migrates it is not easy to confirm which position the fatty acid is esterified in the natural state.

1.2 LIPID COMPOSITION OF RUMINANT DIETS

1.2.1 Forage and grass lipids

Ruminant dietary lipids are predominantly of leaf origin, comprising 6 to 8 % of the dry matter of leaf tissue and are characterised by a high content of glycolipids and phospholipids (Harfoot, 1978). Glycolipids comprise 70-80 % of the lipids present while phospholipids, triacylglycerides and cholesterol esters account for the remaining 20-30 % (Christie, 1978).

Plant lipids are dominated by a high proportion of unsaturated fatty acids especially linoleic acid (C18:2*n*-6) and linolenic acid (C18:3*n*-3), along with smaller amounts of oleic acid (C18:1*n*-9) (Palmquist and Jenkins, 1980). Most of these lipids are present in the chloroplast of leaf tissue, and account for 22 to 25 % of the lipid on a dry weight basis, of which monogalactosyldiglycerides and digalactosyldiglycerides together comprise 80 % of the total lipids (Harfoot, 1978). O'Brien and Benson (1964) determined the fatty acid composition of the monogalactosyldiacylglycerides from lucerne (alfalfa) and found that the fatty acid composition on a weight basis was C16:0, 2.7 %; C18:2*n*-6, 1.7 % and C18:3*n*-3, 95 %. Thus the intake of forage by ruminants consists largely of glycolipids and phospholipids and is virtually devoid of unesterified fatty acids and their triacylglycerides.

Fresh grass contains a high proportion (0.50-0.75 g/g) of the total fatty acids as C18:3*n*-3, the precursor for the longer chain *n*-3 fatty acids (Hawke 1973). The concentration of C18:3*n*-3 varies with the stage of maturity (Bauchart *et al.* 1984) and the grass species (Dewhurst and Schollan, 1997; Table 1.2). Noble (1981) suggested that small but significant losses in C18:3*n*-3, with corresponding increases in C16:0, would occur during drying and storage of forages. Thus grass or lucerne hay would have lower C18:3*n*-3 and higher C18:2*n*-6 than fresh grass or ensiled silage. Dewhurst and Scollan demonstrated that wilting and shading of grass leads to substantial losses of fatty acids particularly C18:3*n*-3 (Table 1.2).

Table 1.2 *Principal fatty acids in different grass species (g/kg DM)*

	Lolium perenne	Lolium multiflorum	Lolium hybrids	Lolium Fescue hybrids	Fescue	Cocksfoot	Timothy	s.e.d	sign
Total fatty acids	22.5	20.8	23.2	23.0	21.9	19.1	21.8	0.83	***
C16:0 palmitic	4.71	4.51	4.90	4.61	4.40	3.99	4.13	0.190	***
C18:2*n*-6 linoleic	2.97	2.73	3.23	3.05	2.55	2.87	3.59	0.136	***
C18:3*n*-3 α-linolenic	11.60	10.52	11.87	12.25	12.06	10.27	11.26	0.671	*
Proportions (g/g)									
C16:0	0.209	0.217	0.212	0.200	0.201	0.209	0.190	0.0093	ns
C18:2*n*-6	0.132	0.137	0.143	0.135	0.116	0.152	0.164	0.0085	**
C18:3*n*-3	0.516	0.503	0.509	0.532	0.551	0.532	0.513	0.0163	*

Data from Dewhurst and Schollan (1997)

Table 1.3 *Effect of wilting and shading on concentrations (g/kg DM) of fatty acid in grass silage*

	Not wilted	Wilted	sed	sign	Not shaded	Shaded	sed	sign
Total fatty acids (g/kg DM)	24.56	17.51	0.648	***	23.09	18.99	1.256	ns
C16:0	5.33	4.69	0.183	ns	5.13	4.89	0.111	ns
C18:2*n*-6	3.08	2.15	0.064	***	2.96	2.27	0.141	*
C18:3*n*-3	13.79	8.34	0.535	***	12.61	9.52	0.977	ns

Data from Dewhurst and King (1998)

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1.2.2 Oilseeds

Ruminant diets are commonly supplemented with high-energy feedstuffs referred to as 'concentrate'. Concentrate feeds consists largely of crushed cereal grains to which are added either crushed oil seeds or their extracts, mostly processed in order to facilitate incorporation and to prevent negative action on fibre digestion in the rumen (Palmquist, 1988). Concentrate supplements are used for different reasons. Firstly to enhance the energy value of diets especially in high yielding animals (Palmquist, 1988). Secondly to manipulate the digestion and absorption of different nutrients e.g. fats can limit rumen acidosis or depressed milk fat content resulting from high carbohydrate or low fibre diets (Palmquist, 1984). Concentrates are also used to manipulate the fatty acid composition of ruminant products making them more desirable for the food industry and for human consumption (Grummer, 1991).

Whereas C18:3*n*-3 comprises a high proportion of the total fatty acids present in grass and forages, commonly used oil seeds contain high proportions of C18:2*n*-6, but a limited number have significant concentrations of *n*-3 fatty acids (Table 1.). Of particular note is linseed oil, with more than 50 % of total fatty acids as C18:3*n*-3 (Sim, 1990). The fatty acid composition of linseed type Linola™ is however different from normal linseed and contains high levels of C18:2*n*-6.

Higher plant oils are completely devoid of any C20:5*n*-3 and C22:6*n*-3, although the development of transgenic plants which express the desaturase gene, resulting in the accumulation of C18:3*n*-3 are currently being investigated. Reddy and Thomas (1996), cloned a cynobacterial Δ^6-desaturase gene, and expressed it in transgenic tobacco resulting in gamma-linolenic acid (GLA, γ-C20:3*n*-6) accumulation. The same technology could be used to produce C20:5*n*-3 and C22:6*n*-3 in higher plants (Kyle *et al.* 1990). Despite the general lack of long chain *n*-3 fatty acids in higher plants, there is some evidence that lower land plants such as the mosses and ferns can contain significant amounts of EPA and certainly have low *n*-6 to *n*-3 ratios (MAFF: Sources of *n*-3 PUFA, 1997). There is no evidence in the literature of attempts to develop mosses and ferns as sources of long chain fatty acids.

Table 1.4 *Fatty acid composition of commonly used fat sources (% of total fatty acids)*

Fatty acid	C16:0	C16:1	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	C20:0	C20:1	C22:0	C22:1
Sunflower	6.0	0.4	5.0	20.0	66.0	0.2	1.0	0.5	1.0	_
Rapeseed	3.5	0.4	1.2	14.2	13.8	9.1	_	10.9	_	46.9
Soybean	11.0	0.2	3.5	22.5	56.0	7.5	1.2	0.4	0.5	_
Corn	11.0	1.0	2.0	28.0	58.5	2.0	1.0	0.5	0.5	_
Safflower	6.5	0.2	2.0	76.5	16.0	0.2	0.3	0.2	0.2	_
Linseed	5.0	0.5	2.5	16.5	15.0	63.5	0.5	0.6	_	_
Borage	12.0	0.4	5.0	17.0	35.0	21.0*	0.4	3.0	_	3.0
Cottonseed	24.0	1.0	2.0	17.0	47.0	1.0	_	_	_	_
Palm oil	44.5	0.3	5.0	39.0	10.5	0.4	0.4	_	_	_
Tallow	25.0	3.3	29.0	39.5	2.7	0.5	0.15	0.1	_	_

* γ-Linolenic acid ; *From Oils and Fats Manual A. Karleskind 1996.*

Table 1.5 *Fatty acid composition of various fish oils (g /100 g total fatty acids)*

Fatty acids	Farmed salmon	*Composition of oil fed to salmon*	Wild salmon flesh	Herring	Capelin	Anchovy
C14:0	5	5	4	7	7	7
C16:0	16	15	13	13	13	17
C16:1n-7	7	6	4	7	10	9
C18:0	3	2	3	1	1	4
C18:1n-9	13	10	19	10	14	12
C18:2n-6	3	3	1	1	2	1
C18:3n-3	2	2	1	1	1	1
C18:4n-3	3	5	1	3	4	_
C20:1n-9	9	9	12	13	16	2
C20:5n-3	7	9	7	6	8	17
C22:1n-11	10	15	11	23	15	2
C22:6n-3	12	12	10	6	6	9
Saturates	24	22	20	21	21	28
Monounsaturate	43	42	52	53	55	23
n-3 PUFA	28	30	24	16	19	31

PUFA, polyunsaturated fatty acids; Data from Sargent, (1997)

1.2.3 Fish oil

The fatty acid composition of marine organisms contrasts markedly with that of higher plants and contains substantial concentrations of C20:5*n*-3 and C22:6*n*-3 (Nettleton, 1991). Fish oil contains higher concentrations of unsaturated fatty acids than land animals because fish like monogastrics, absorb and deposit the wide range of fatty acids present in their diet (Table 1.5). This transfer of fatty acids through the marine food web has been reviewed by Sargent and Henderson, (1995) and Sargent *et al.* (1995). As discussed by Sargent (1996), the fatty acid composition of marine zooplanktons largely reflects that of the phytoplanktons on which they depend for food. Marine zooplanktons can produce and deposit a range of fatty acids, including long chain monounsaturated acid (C20:1*n*-9 and C22:1*n*-11), that are not present in phytoplankton (Table 1.5). The oil reserves of zooplanktons can either be triacylglycerides or wax esters (fatty alcohols esterified to fatty acids). Wax esters are deposited as an adaptation to extreme light regimes in the polar latitudes that determine the sharp and limited seasonal availability of phytoplanktons (Sargent, 1997). Under such conditions the zooplankton can accumulate more than 50 % of their dry weight as oils composed of wax esters, while zooplanktons in lower latitudes accumulate much lower levels of oils containing negligible levels of wax esters and high levels of phospholipids.

Fish (higher altitude fish e.g. herring, capelin, mackerel and sprats) consuming zooplanktons containing wax esters, digest, assimilate and convert the waxes to triacylglycerol. High latitude fish thus contains high levels of C20:1*n*-9 and C22:1*n*-11 and relatively low levels of *n*-3 PUFA, whilst the content of C20:5*n*-3 often exceeds that of C22:6*n*-3 (Table 1.5) (Sargent, 1997). In contrast fish consuming zooplanktons in the lower latitudes (sardines, pilchards and anchovy), have lower body triacylglycerides and consequently higher percentages of *n*-3 PUFA than high latitude fish oils, with C20:5*n*-3 exceeding C22:6*n*-3 (Table 1.5).

1.3 LIPID METABOLISM IN RUMINANTS

1.3.1 Lipid digestion in the rumen

Dietary lipids on entering the rumen are subjected to hydrolysis by microbial lipases after which unsaturated fatty acids are extensively biohydrogenated (Harfoot, 1978 and Noble, 1978). The hydrolysis of the glycerol fatty acid ester bond is a prerequisite to biohydrogenation of the fatty acids which is accomplished via isomerization and reduction reactions (Figure 1.7) (Hawke and Silcock, 1969). Because of these microbial transformations, the fatty acid composition of rumen lipids and post-ruminal digesta, differs significantly from that present in the diet.

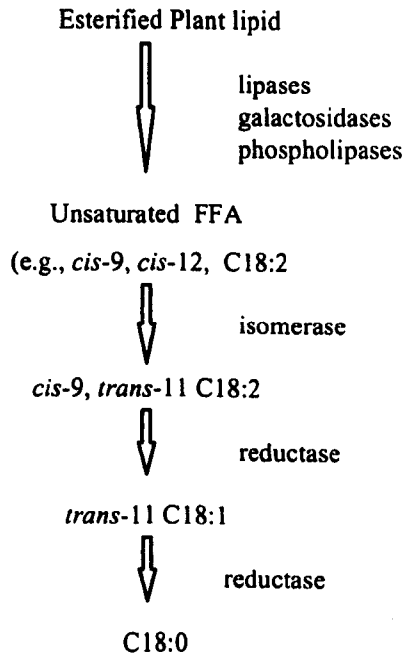


Figure 1.7 Key steps in the conversion of esterified plant lipid to saturated fatty acids by lipolysis and biohydrogenation in ruminal contents

(Adapted from Jenkins, 1993)

1.3.2 Hydrolysis of dietary lipids

Shortly after ingestion, esterified dietary lipids are hydrolysed extensively by microbial lipases, causing the release of constituent fatty acids and glycerol which is rapidly fermented, yielding propionic acid as a major end product (Hazelwood and Dawson, 1976). The hydrolytic activity of rumen microorganisms was first demonstrated by Garton *et al.* (1959) who incubated linseed and olive oils with rumen contents from sheep. Subsequent

investigations have shown that this hydrolytic activity extends to a wide range of esterified substrates, which may or may not be considered as natural constituents of the diet. Rumen hydrolysis has been demonstrated not only with mono- and digalactosylglycerides (Dawson and Hemington, 1974), phospholipids (Hazlewood *et al.* 1976), and a wide range of triacylglycerides (Moore *et al.* 1969), but also with substrates such as sterol esters, methyl esters and ethyl esters. Under normal circumstances the hydrolysis of triacylglycerides in the rumen is both rapid and complete with little accumulation of mono- or diacylglycerides (Hawke and Silcock, 1970).

Lipolytic bacteria, with potential to hydrolyse the different lipid components of the diet have been isolated from the rumen contents using anaerobic techniques (Henderson, 1973; Hazlewood and Dawson, 1975 and 1979). *Anaerovibrio lipolytica*, which is best known for its lipases activity, produces cell bound esterase and lipase enzymes (Henderson, 1971). Lipase activity is entirely extracellular, being associated with the cell-surfaces or extracellular membranous structures composed of protein, lipid and nucleic acid (Henderson, 1973). The optimal activity for lipase from *anaerovibrio lipolytica* is at pH 7.4. Devendra and Lewis (1974a) observed that lipase activity was enhanced by $CaCl_2$ and $BaCl_2$, and suggested that cations were required for microbial function. Lathman *et al.* (1972), reported that triacylglycerides were rapidly hydrolysed by lipases characteristic of *Anaerovibrio lipolytica*, while diacylglycerides were hydrolysed more rapidly by lipases from *Butyrivibrio fibrisolvens* (Prins *et al.* 1975).

Hazelwood and Dawson (1979) isolated fatty acid autotrophic bacteria (*Butyvibrio*, strain S2) which hydrolysed and hydrogenated dietary long chain fatty acids prior to incorporation into membrane lipids. The optimal activity of *Butyvibrio* (strain S2), was at pH 6.5-7.5, and its activity was inhibited by aerobic conditions (Hazelwood and Dawson, 1975). This strain of bacteria was later shown to posses the enzymes phospholipase A, phospholipase C, lysophospholipase and phosphodiesterase enzymes, which hydrolysed plant phospholipids (Hazelwood *et al.* 1983), whilst a variety of galactosidases hydrolysed plant galactolipids. According to Bauchart *et al.* (1990), the extent of rumen hydrolysis is high for most unprotected lipids (85 to 95 %) and this percentage is higher for diets rich in fats than in diets where most lipids are in the cellular lipids (i.e. grass). *In vitro* (Noble *et al.* 1974), *in vivo*

(Demeyer and Van Nevel, 1995) and *in sacco* (Perrier *et al.* 1992), measurements of lipid disappearance show that hydrolysis is rapid irrespective of the diet.

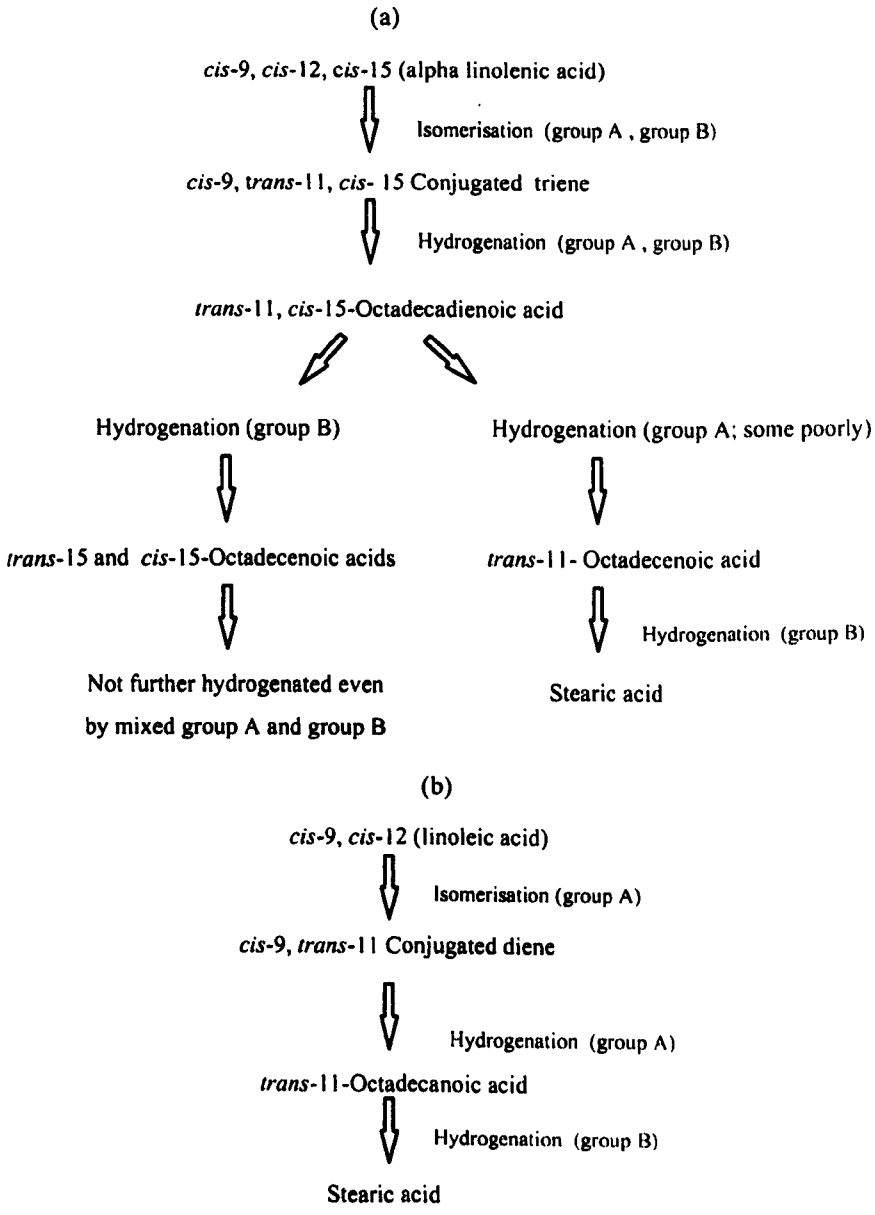
Hydrolysis of dietary lipids is often low at low rumen pH, for example when animals are on high grain or concentrate diets (Ben Salem *et al.* 1993). *In vitro* studies by Henderson *et al.* (1969), using lipase from *Anaerovibrio lipolytica*, demonstrated that lipase activity was severely inhibited at pH 5.8, but this effect could be avoided by maintaining the pH above 6.3. Russell and Dombrowski (1980) attributed the observed negative effects of low pH on rumen hydrolysis to the growth of *Anaerovibrio lipolytica* (the major rumen lipolytic bacteria), which decreases at low rumen pH 5.7, and is completely inhibited at pH 5.3. Similarly, the hydrolytic activity of *Butyrivibrio fibrisolvens* (lipolytic and hydrogenating species), was 75 % of its maximum yield at pH 5.75, but was completely washed out of continuous culture at pH 5.5 (Russell and Dombrowski, 1980). In another study using olive oil, lipase activity was highest at pH 7.4, while at pH 6.6 only 50 % of the maximum activity was observed (Henderson, 1971).

1.3.3 Rumen biohydrogenation

Over 50 years ago, Banks and Hilditch (1931), observed that the tissue lipids of ruminants were more saturated than those of non-ruminants and concluded that biohydrogenation of forage lipids took place in ruminant tissues. Since then it has been demonstrated that C18 unsaturated fatty acids, liberated from glycerol esters by lipolysis are biohydrogenated by rumen microbes to more saturated fatty acids predominantly stearic (C18:0) and vaccenic acid (*trans*-11, C18:1) (Figure 1.8), which are later absorbed and deposited into ruminant tissues (Noble, 1978).

The biohydrogenation of C18:3*n*-3, proceeds according to the scheme shown in Figure 1.8a whilst that of C18:2*n*-6 is shown in Figure 1.8b. Both pathways involve an initial isomerisation step resulting in the formation of a conjugated *cis*-9, *trans*-11 acid from the *cis*-12 double bond, in unsaturated C18 fatty acids. This isomer then undergoes hydrogenation of its *cis* double bonds leaving the *trans*-11 octadecenoic acid (*trans* vaccenic acid, 11-elaidic acid) as an intermediary product which is finally hydrogenated to stearic acid. The biohydrogenation pathways have been demonstrated by Noble *et al.* (1969) who incubated unsaturated fatty acids in rumen contents *in vivo*, Dawson and Kemp, (1970) who incubated

unsaturated fatty acids in rumen contents *in vitro* and Verhulst *et al.* (1985), incubated unsaturated fatty acids in pure bacterial cultures.



(Group A and group B refers to classes of biohydrogenating bacteria)

Figure 1.8 Scheme for the biohydrogenation of α-linolenic acid and linoleic acid (Adapted from Harfoot and Hazlewood, 1988)

1.3.4 Rumen biohydrogenators

Rumen biohydrogenating bacteria can be divided into two distinct groups A and B (Kemp and Lander, 1984). Members of group A mostly hydrogenate C18:2*n*-6 and C18:3*n*-3 to *trans*-11 C18:1, (for example the *Butyrivibrio* species). However, some isolates produce *trans*-11, *cis*-15-C18:2 from C18:3*n*-3 (Hazelwood *et al.* 1976). Group A bacteria appear to be incapable of hydrogenating C18:1 acids to C18:0. Members of group B bacteria (for example *Fusocillus* species) biohydrogenate a wide range of C18:1 acids, including C18:1*n*-9 and *trans* 11-C18:1 (*trans* vaccenic acids), as well as C18:2*n*-6 to C18:0. Hazelwood *et al.* (1976) suggested that group A bacteria, consist of three distinct populations: Group 1 bacteria, which were capable of hydrogenating only the *cis*-double bond of the *cis*-9, *trans*-11 conjugated diene derived from C18:2*n*-6, yielding *trans*-11 monoenoic as the end product (Figure 1.3.2). Group 2 bacteria hydrogenated the *cis*-15 double bond of the *cis*-9, *trans*-11, *cis*-15 conjugated triene derived from C18:3*n*-3, and Group 3 bacteria which correspond to group B bacteria identified by Kemp and Lander (1984), hydrogenated a wide range of C18 fatty acids to C18:0 (Figure 1.3.2). The complete biohydrogenation of C18:3*n*-3 and C18:2*n*-6 to C18:0 thus takes place only in the presence of both members of group A and B (Kemp and Lander, 1984). Once the *cis*- or *trans*-15 monoenoic acids are formed (Figure 1.3.2a), they are not biohydrogenated by any members of either groups (Body, 1976), for reasons not well understood. For a high yield of C18:0 *in vitro* from C18:3*n*-3, it was necessary to strike a balance between the number of each bacterium in the culture (Kemp and Lander, 1984).

The problem of how the *trans*-11 C18:1 is transferred between the groups A and B is not well understood. However, Harfoot *et al.* (1973), observed that the conversion of C18:2*n*-6 into, *trans*-11 C18:1 and subsequently into C18:0 was largely associated with food particles. The authors suggested that because hydrolysis of esterified fatty acids in the rumen was extracellular, and hydrolysis was a prerequisite for biohydrogenation (Kemp *et al.* 1984), biohydrogenation was also an extracellular process. Hawke and Silcock, (1970) had earlier observed a decrease in biohydrogenation, when strained rumen liquor was centrifuged and attributed the decrease to the removal of biohydrogenating bacteria adhered to food particles. It may be possible that adsorption onto feed particles plays a role in biohydrogenation, but at present there is no conclusive evidence that biohydrogenating enzymes are extracellular.

1.3.5 Factors affecting biohydrogenation

In a review of the literature (Doreau and Ferlay, 1994) the percentage biohydrogenation of C18:3*n*-3 ranged between 85 to 100 %, while that of C18:2*n*-6 ranged between 70 to 95 %, (Figure 1.9). Kemp *et al.* (1984) suggested that biohydrogenation was a mechanism for detoxifying highly toxic unsaturated fatty acids and as such has a survival value, not only to the rumen microbial ecosystem in general but also for the ruminant animal. The reticuo-rumen provides a continous culture for anaerobic bacteria and protozoa responsible for the breakdown of cellulose and other resistant polysaccharides. Besides contributing to the energy supply of the ruminant, it ensures that other nutrients, which might escape digestion, are exposed to enzyme action post ruminally. Thus, the more highly bacteriostatic fatty acids are hydrogenated faster than the less toxic fatty acids, for example the biohydrogenation C18:3*n*-3 is higher than that of C18:1*n*-9 which is less toxic (Dawson and Kemp, 1970).

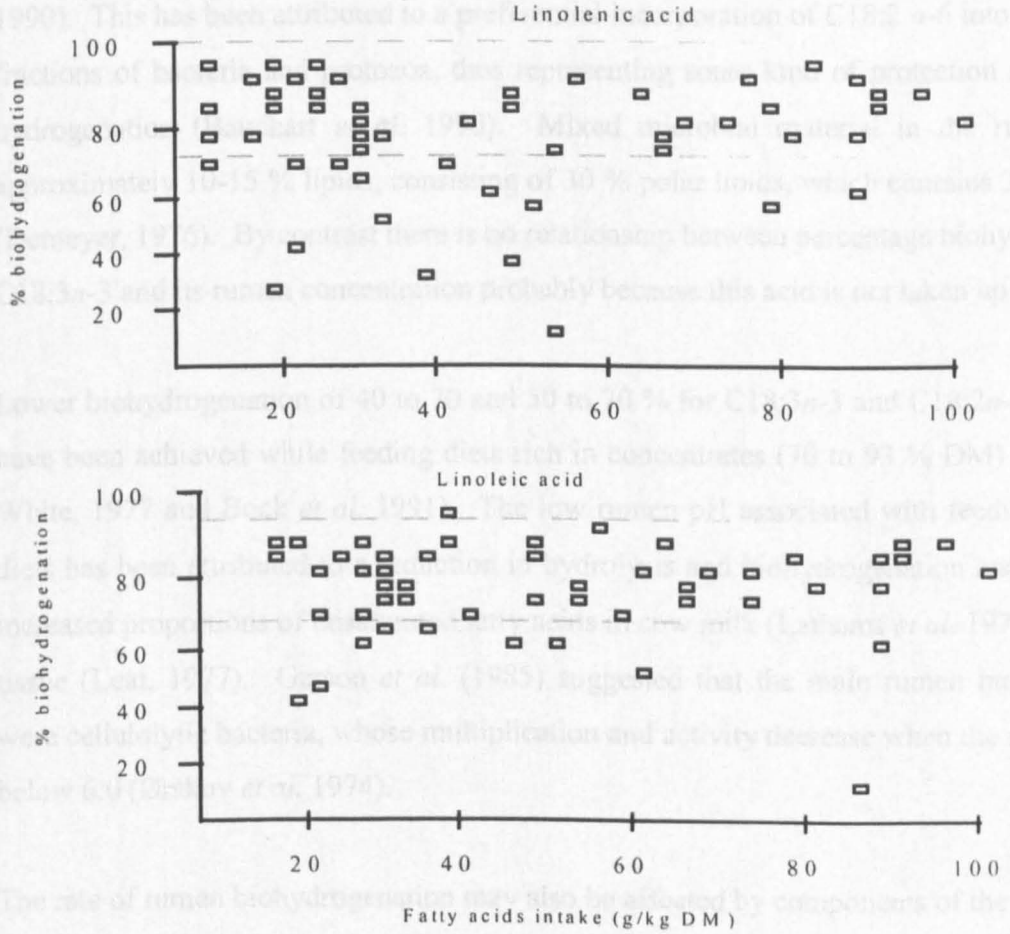


Figure 1.9 Ruminal biohydrogenation of dietary linolenic and linoleic acids

(Adapted from Doreau and Ferlay, 1994)

The extent of biohydrogenation depends on whether the substrate is added as free acid or triacylglycerides and to some extent on whether the experiment carried out *in vitro* or *in vivo*. Noble *et al.* (1969) observed that when emulsions of trilinoleinic were infused into the rumen, the C18:2*n*-6 released by hydrolysis was hydrogenated completely to C18:0, whereas when comparable amounts of free acid were infused, hydrogenation proceeded only as far as the *trans*-11 C18:1 intermediate. Similar observations were reported by Harfoot *et al.* (1975), that where free acids inhibited complete biohydrogenation in *in vitro* system. Henderson, (1973) noted that the inhibitory effects of fatty acids occurred when the amount of fatty acid present was greater than could be adsorbed onto bacterial cells. Kemp *et al.* (1984) later observed that feed particles rapidly adsorbed fatty acids thus reducing their toxic action on rumen bacteria.

The levels of C18:2*n*-6 escaping biohydrogenation are relatively constant, such that biohydrogenation was low when C18:2*n*-6 concentration in the diet was low (Bauchart *et al.* 1990). This has been attributed to a preferential incorporation of C18:2 *n*-6 into the polar lipid fractions of bacteria and protozoa, thus representing some kind of protection against further hydrogenation (Bauchart *et al.* 1990). Mixed microbial material in the rumen contains approximately 10-15 % lipids, consisting of 30 % polar lipids, which contains 30 % C18:2*n*-6 (Demeyer, 1976). By contrast there is no relationship between percentage biohydrogenation of C18:3*n*-3 and its rumen concentration probably because this acid is not taken up by bacteria.

Lower biohydrogenation of 40 to 70 and 50 to 70 % for C18:3*n*-3 and C18:2*n*-6 respectively, have been achieved while feeding diets rich in concentrates (70 to 93 % DM) (Faichney and White, 1977 and Bock *et al.* 1991). The low rumen pH associated with feeding concentrate diets has been attributed to a reduction in hydrolysis and biohydrogenation and consequently increased proportions of unsaturated fatty acids in cow milk (Lathams *et al.* 1972) and adipose tissue (Leat, 1977). Gerson *et al.* (1985) suggested that the main rumen biohydrogenators were cellulolytic bacteria, whose multiplication and activity decrease when the rumen pH falls below 6.0 (Ørskov *et al.* 1974).

The rate of rumen biohydrogenation may also be affected by components of the diet other than the lipid composition. Gerson *et al.* (1985) observed that the rates of lipolysis and biohydrogenation decreased significantly with decreasing dietary fibre and this was reflected in the decreasing amounts of C18:0, resulting from C18:2*n*-6 in the rumen digesta. When

different concentrations of dietary nitrogen were added to diets of constant digestible organic material, unsaturated fatty acids comprised between 70 to 80 % of the esterified fatty acids in the rumen with 1.1 to 1.4 % dietary nitrogen (Gerson *et al.* 1982). This decreased to 20 to 30% when low (0.5 %) and very high nitrogen (3.4 %) nitrogen was present. It was subsequently observed that increased dietary nitrogen was accompanied by increased rates of hydrolysis and biohydrogenation *in vitro* (Gerson *et al.* 1983), thus confirming earlier suggestions that microbial population was important in controlling hydrolysis and biohydrogenation. This is in agreement with recent findings of decreased hydrolysis at low rumen pH (Van Nevel and Demeyer, 1996a) and decreased biohydrogenation through antibiotic use (Van Nevel and Demeyer, 1995).

1.4 MICROBIAL LIPIDS

Rumen microbial population includes bacteria, ciliate protozoa, flagellate protozoa, phycomycete fungi, amoebae and bacteriophages (Harfoot and Hazelwood, 1989). Microbial lipids contribute approximately 10-15 % by weight of the total lipids available in the post-ruminal digesta (Wu *et al.* 1991). Mixed rumen bacterial lipids consist of 30 % phospholipids and 70 % non-phospholipids, of which greater than 40 % are unesterified fatty acids (Viviani *et al.* 1968). The phospholipids consisted of phosphatidyl choline (1.2 %), phosphatidylethanolamine (66.6 %) and phosphatidylserine (21.6 %) together with unidentified polar lipids (11 to 12 %).

1.4.1 Bacteria

Rumen bacterial lipids, in contrast to total rumen lipids or forage lipids, are characterised by a high proportion of straight odd chain and branched chain saturated fatty acids. Ferlay *et al.* (1993) demonstrated *in vivo* that rumen microorganism could incorporate and synthesise long chain fatty acids of different chain lengths (carbon 15, 16, 17 and 18). Rumen bacteria also take up and incorporate exogenous long chain fatty acids especially C18:2*n*-6 into cell vacuoles (Demeyer *et al.* 1978; Emmanuel, 1978 and Bauchart *et al.* 1990). Some bacterial populations characteristic of the genus *Butyrivibrio*, present an absolute requirement for long chain fatty acid for growth (Hazelwood and Dawson, 1977 and 1979).

Lipid supplementation increases the fatty acid content of solid-adherent and liquid associated bacteria (Legay-Carmier and Bauchart, 1989; Bauchart *et al.* 1990; O'Kelly and Spiers, 1991). These increases in bacterial lipids were correlated with the level of dietary fat (Weisberg *et al.* 1992; Klusmeyer *et al.* 1991b). Bauchart *et al.* (1990) and Czerkawski, (1986) also observed that the proportion of lipids in solid associated bacteria was greater than in liquid associated bacteria. This was attributed to a selective attachment of bacteria of a specific composition, or to a higher incorporation of free fatty acids preferentially adsorbed onto particulate matter (Harfoot, 1981). Emmanuel (1978), reported a partial inhibition of bacterial *de novo* lipid synthesis by dietary long chain fatty acids, which was associated with the reduced substrate availability, resulting from a decrease in volatile fatty acid production (Kurihara *et al.* 1978) while feeding diets with added fat. Thus the large increases in C18:2*n*-6 in solid adherent bacteria, reported by Bauchart *et al.* (1990), was probably due to a preferential incorporation

of this acid rather than synthesis of C18:2*n*-6 by rumen bacteria. This hypothesis can be supported by two observations. First the lack of convincing evidence in the literature for the synthesis of C18:2*n*-6 or C18:3*n*-3 by pure cultures of rumen bacteria (Demeyer *et al.* 1978). Secondly the large increases in microbial fatty acids proportional to increases in dietary fat (Bauchart *et al.* 1990).

1.4.2 Protozoa

Large variations in the composition and suspension density of rumen protozoal population occur both within and between animals (Coleman, 1980). This is mainly a result of technical difficulties involved in obtaining protozoal samples uncontaminated by bacteria and feed particles as well as a lack of uniformity in analytical procedures. Results obtained by Harfoot, (1978), under defined conditions indicate that mixed protozoa contain a high proportion of phospholipid (85.5 %) and only small quantities of mono-, di- and tri-acylglycerides. Total fatty acids were less saturated than those of mixed rumen bacteria and contained roughly equal amounts of C18:1*n*-9 (18%) and C18:2*n*-6 (16%) and a much smaller amount of C18:3*n*-3 (less than 1%).

Lipid supplementation also increases the fatty acid content of protozoa, by 50 to 100 % (Bauchart *et al.* 1986 and Weisbjerg *et al.* 1992). This has been attributed to direct incorporation of exogenous fatty acids by protozoa, which is greater than *de novo* synthesis (Demeyer *et al.* 1978). The extent of exogenous fatty acids incorporation depends on the nature and concentration of the fatty acids. For example soybean oil increases C18:2*n*-6 in the free fatty acid fraction but not in the phospholipid fraction while C18:0 increases with tallow supplementation which is high in C16:0 and C18:1*n*-9 (Weisbjerg *et al.* 1992).

1.4.3 Biosynthesis of microbial lipids

Harfoot (1978) has reviewed aspects of microbial *de novo* fatty acids biosynthesis. Microbial lipids are synthesised by chain elongation of branched-chain precursors generated by metabolism of branched-chain amino acids (Garton, 1977; Figure 1.10). In addition mixed bacteria are able to synthesise long-chain fatty acids from acetate and glucose (Emmanuel *et al.* 1978). Fatty acids synthesised *de novo* consist mainly of C18:0 and C16:0 in an approximate ratio of 2:1 (Knight *et al.* 1978).

Monounsaturated fatty acids constitute 15 to 20 % of bacterial fatty acids, and are synthesised by the anaerobic pathway (Fulco, 1983). In this pathway (Figure 1.10) the β-hydroxy C10 intermediate in fatty acid synthesis are dehydrated in β & γ position forming a *cis*-3 double bond instead of a *trans*-2 double bond. Reduction of the bond in this position cannot occur and hence the double bond is maintained through the elongation and subsequent turns of the cycle. Single bonds may also be formed by the action of an anaerobic desaturase on stearic acid (Hazelwood and Harfoot, 1988). Polyunsaturated fatty acids are not commonly synthesised by bacteria.

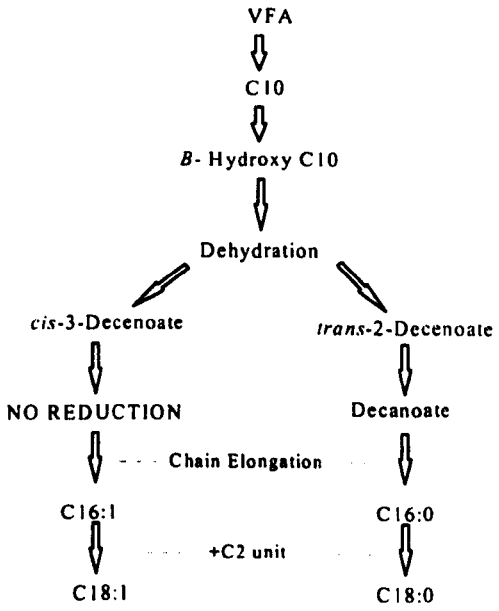


Figure 1.10 Biosynthesis of microbial lipids

(Adapted from Fulco, 1983)

1.5 EFFECTS OF DIETARY FAT ON RUMEN METABOLISM

1.5.1 Digestion and fermentation

Dietary fats in ruminant diets can greatly disrupt fermentation in the rumen, causing reduced digestibility of non-lipid energy sources. Rumen digestion of structural carbohydrates was reduced by 50 %, when diets with more than 10 % added fat were fed to sheep (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984). Dietary fat is however less detrimental to non-structural carbohydrate digestion, and normal starch digestion has been reported in the rumen of cattle offered diets with additional fat, even though the dry matter and fibre digestibility were depressed (Zinn, 1988). The reduction of rumen fermentation is accompanied by a reduced production of methane, hydrogen and volatile fatty acids including lower acetate to propionate ratio (Chalupa *et al.* 1984).

Rumen disturbances are greater with unsaturated fatty acids (Chalupa *et al.* 1984) especially linseed oil which is rich in C18:3*n*-3 (Ikwuegbu and Sutton, 1982 and Sutton *et al.* 1983), compared to saturated fatty acids (Table 1.6). In addition, a free carboxyl group is important in the inhibition of fermentation. Hence free fatty acid derivatives such as calcium salts of long chain fatty acids (Jenkins and Palmquist, 1982), fatty acyl amides (Fotouhi and Jenkins, 1992) and triacylglycerides (Chalupa *et al.* 1984) all inhibit rumen metabolism to a lesser extent than the free fatty acids. Once ingested the inhibitory effects of unsaturated fatty acids are regulated by the rates of rumen hydrolysis and biohydrogenation. The degree of carboxylate salt formation in the rumen may also affect the extent to which dietary fats affect rumen fermentation (Jenkins and Palmquist, 1982; Palmquist *et al.* 1986). High levels of triacylglycerides in the diet increase the total lipid content of the rumen, but the concentration of unsaturated fatty acids maybe low when the rate of hydrolysis and biohydrogenation are low (Jenkins, 1993).

Less negative effects on rumen fermentation have been observed while feeding diets rich in hay (Devendra and Lewis, 1974*b* and Ben Salem *et al.* 1993). Mir, (1988), offered sheep ground alfalfa hay supplemented with 10 % rapeseed oil (canola) without any detrimental effects on ruminal volatile fatty acid pattern. Doreau *et al.* (1991) found no effect of 10 % rapeseed oil or tallow on ruminal organic matter digestion when dairy cows were fed basal diets of 50 % hay although the volatile fatty acid pattern was altered. Substitution of alfalfa

hay with corn silage in dairy diets increased the benefits of whole cottonseed by increasing dry matter intake and milk yield (Smith *et al.* 1993), demonstrating a significant interaction between dietary fat and fibre. In contrast, Klusmeyer *et al.* (1991), failed to observe any advantage of dietary fibre while feeding ruminally inert calcium salts as fat sources.

Table 1.6 Digestion and microbial synthesis in the rumen of sheep given diets supplemented with free and protected oils

Treatment	Basal diet	Linseed oil	Protected linseed oil	Coconut oil	Protected coconut oil	sem
OM (g/d)						
Food	490	532	537	529	531	2.3
Duodenum	255	*378	*333	*377	*299	8.4
Faeces	116	*142	128	*152	126	4.2
NDF (g/d)						
Food	199	200	199	198	199	0.5
Duodenum	99	*160	*141	*172	*124	7.6
Faeces	68	*94	*83	*106	*84	2.9
Molar proportion (mol/mol total VFA)						
Acetic acid	0.651	*0.542	*0.615	*0.521	0.642	0.008
Propionic acid	0.179	*0.333	*0.237	*0.374	0.211	0.009
n-Butyric acid	0.127	*0.082	*0.093	*0.076	*0.105	0.006
Total N						
Food	12.87	12.70	12.97	12.78	12.91	0.087
Duodenum	15.41	18.81*	16.50	19.90*	14.68	0.865
Faeces	3.61	3.66	3.23	3.60	3.14	0.128
N flow in duodenal digesta						
Ammonia-N	1.17	1.14	1.32	1.15	1.19	0.085
Non-ammonia N	14.24	17.67*	15.18	18.75*	13.75	0.827
Microbial N						
DAPA	7.07	12.75*	9.20	10.18*	8.42	0.718
RNA	9.54	13.90	13.00	12.44	10.28	1.230

*Differences between the supplemented diets and the basal diet were significant; Summarised from Sutton *et al.* (1983)

Devendra and Lewis, (1974), proposed several mechanisms which explain the inhibitory effects of lipids on rumen fermentation. Among them were the 'lipid coating' and 'antimicrobial' theories. The lipid coating theory, attempts to explain the physical-chemical

nature of the inhibition mechanism, and stems from the observation that fatty acids adhere to feed particles and microbial cells thus impending the passage of essential nutrients and attachment of cellulase to cellulose (Henderson, 1974). This theory thus relates the reduction in fermentation to a lipid layer over feed particles that inhibit cellulose digestion. Harfoot *et al.* (1974), also observed that bacteria grown in culture adsorbed more than 90 % of added fatty acids until feed particles are added, then 60 % or more of the fatty acids become associated with feed particles. Thus when dietary fibre is high the added lipid is spread over a wider surface area so that the physical coating effect of lipids on feed particles is much less than with diets with low fibre. This explanation is consistent with the observation that more fibre was digested at higher levels of inclusion with the same level of added fat (Devendra and Lewis, 1974). The physical coating of feed particles by added fat in the rumen also reduces the surface area for microbial contact, demonstrating a direct antimicrobial effect of lipids on rumen fermentation, (Galbraith *et al.* 1971 and Henderson, 1973). The extent to which the physical coating occurs probably determines the magnitude of fibre digestion, which is dependent on the level of fibre in the diet.

Henderson (1974), observed in an *in vitro* study that the activity of rumen bacteria contributing to propionate production was not inhibited by fatty acids. Kobayashi and Itabashi (1986), demonstrated that high levels of propionate in the rumen were toxic to rumen protozoa, especially the holotrichs and large entodiniomorphid ciliates. Rowe *et al.* (1985), had earlier demonstrated that butyrate production from acetate increased in defaunated animals as the bacterial population increased ($r^2 = 0.76$). The latter observation was attributed directly to an increased bacterial substrate competition, rather than the direct effect of rumen defaunation. This would explain the consistent *in vivo* observation of increased propionate to acetate ratio in the rumen (Table 1.6) with added dietary fat (Chalupa *et al.* 1984 and Ikwuegbu and Sutton, 1982).

Other hypothesis suggests that the negative effects of lipids on cellulolytic bacteria are probably greater with diets rich in starch where microbial ecosystem and the low rumen pH promote an amylolytic flora and reduces fibre digestion (Sukhija and Palmquist, 1990; Chalupa *et al.* 1984 and Jenkins and Palmquist, 1982). Jenkins, (1993), compared the antimicrobial effects of lipids on rumen microorganisms to the cytotoxic effects of fatty acids on membrane functions of eucaryotic cells, such as uncoupling of oxidative phosphorylation.

Long chain unsaturated fatty acids readily attach to the lipid bilayer in biological membranes because of their hydrophobic and amphiphilic nature. The negative effects of these amphiphiles are associated with their ability to melt, expand, thicken and fluidize functionally important lipid phases near membrane proteins. Lipids in the rumen may similarly inhibit fermentation by partitioning into microbial plasma membranes and hence disrupting microbial functions. This may explain the observed inhibited amino acid uptake and energy metabolism in bacterial protoplasm in the presence of exogenous lipid (Galbraith and Miller, 1973).

1.5.2 Microbial synthesis and efficiency

The efficiency of microbial synthesis in the rumen is mainly determined by the balance between synthesis and degradation of microbial matter, the latter process being the result of predatory activity of protozoa towards bacteria (Demeyer and Van Nevel, 1995). Theoretically, synthesis of microbial biomass is related to the amount of ATP produced in the rumen through fermentation of organic matter. Efficiency is then expressed as grams of nitrogen incorporated per kilogram of organic matter (OM) apparently digested in the rumen (microbial N/kg OMADR) or as organic matter truly degraded in the rumen (microbial N/kg OMTDR). This is the difference between OM intake and OM entering the duodenum (Czerkawski, 1986).

An increased efficiency of microbial protein synthesis has been reported when feeding full-fat rapeseed in milking cows (Murphy *et al.* 1987) and in sheep offered diets containing linseed oil (Ikwuegbu and Sutton, 1982; Sutton *et al.* 1983; Table 1.6). However, microscopic examination revealed that protozoa were eliminated from the rumen ecosystem, which may have contributed to the increased efficiency of microbial growth (Lindsay and Hogan, 1972). Leng *et al.* (1978) suggested that protozoa reduced the availability of nutrients (principally protein) to the host animal, by engulfing bacteria and small feed particles which were preferentially retained in the rumen and as such defaunation resulted in increased microbial efficiency.

Defaunation does not always result with increased flow of microbial matter, because fermentable organic matter can be limited by the absence of protozoa (Jouany *et al.* 1988). In a review of the literature by Doreau and Ferlay (1995), the only factor explaining an increase in protein degradation was the decrease in protozoa number subsequent to lipid

supplementation, which involved an increase in bacterial biomass and a higher proteolytic activity (Ushida *et al.* 1991). This hypothesis is consistent with the data of Ikwuegbu and Sutton, (1982), where an increase in dietary nitrogen flow at the duodenum corresponded to defaunation when feeding large amounts of linseed. Doreau *et al.* (1993) however did not observe any difference in microbial synthesis (estimated by ammonia uptake) or efficiency (calculated by the ratio of ammonia uptake to organic matter fermented), when starch was fermented alone or in presence of rapeseed oil, in an *in vitro* system.

The efficiency of microbial synthesis, plotted against dietary fat was significantly increased by supplemental (Doreau and Ferlay, 1995). This was attributed to the decrease in organic matter digestion in the rumen and was significant with dietary saturated and monounsaturated fat, whereas it was not significant when feeding dietary polyunsaturated fatty acids.

1.6 PROTECTED LIPID SUPPLEMENTS

Several technological treatments are used to 'protect' dietary fatty acids against ruminal microorganisms, or to protect ruminal fermentation against the negative effects of dietary lipids (Ashes *et al.* 1979). These treatments include encapsulation of lipids in a formaldehyde-treated protein coat, and formaldehyde treatment of whole seeds, hydrogenation or saponification of fatty acids and crystallization of fat (Figure 1.11). The inclusion of oil seeds instead of oils in ruminants' diets can also offer natural protection.

The essential features of a biologically effective protected lipid supplement are; the supplement should be inert in the rumen; the protection mechanism should be reversible post ruminally and the lipid should be absorbed in the small intestines. Several methods are now available for routine testing of the effectiveness of protected lipid supplements and have been reviewed and discussed extensively by Ashes *et al.* (1979). Using *in vivo* test protection in the order of 75 %, while with *in vitro* test, a 60 % protection is considered satisfactory (McDonald and Scott, 1977).

1.6.1 Formaldehyde protein-oil supplements

Formaldehyde protected lipid supplements were first developed about 30 years ago by Scott *et al.* 1970). The process involved the emulsification of polyunsaturated vegetable oils with casein or plant proteins (pH 6.8 at 70°C)(Figure 1.11a). The emulsion was spray dried and formalin was introduced as a fine mist during the drying process. Inter and intramolecular methylene linkages resulting from the interaction of formaldehyde with amino groups, rendered the protein much less susceptible to ruminal degradation. Upon feeding the proteins were solubilized in the abomasum and the lipid hydrolysed by pancreatic lipase before absorption in the small intestines.

Protected lipids stimulated extensive research into lipid utilisation by ruminants and clearly demonstrated the capability of animals to respond to high levels of dietary fats, without deleterious effects on rumen fermentation (Palmquist and Jenkins, 1980). Ashes *et al.* (1979), observed that *in vitro* the proportion of rumen protection was closely related to the concentration of added formalin and *in vivo* to the milk fatty acid response in goats after feeding the protected supplement. These authors reported a significant correlation (r^2= 0.96,

n=6), between resistance to ruminal hydrolysis and resistance to ruminal biohydrogenation. Scott and Cook (1973), modified the formaldehyde treatment procedure of vegetable oils so that natural oilseeds could be used as sources of both oil and protein. However, some oilseeds, for example sunflower were low in protein, which is required for efficient emulsification and subsequent protection of polyunsaturated oils. This deficiency was overcome by adding small amounts (5 to 10 %) of casein or by mixing soybean seeds or meal with the sunflower seeds (usually 30 to 70 parts, respectively). Oilseeds directly treated with formalin were however only partially protected from rumen hydrolysis and biohydrogenation (Knight *et al.* 1978). This was attributed to the physical breakdown of the product during mastication and sometimes due to insufficient control of the manufacturing process (Ashes *et al.* 1979).

Despite the lack of complete protection, supplementing the diets of steers with formaldehyde protected cotton and canola seeds increased unsaturation in the subcutaneous adipose tissue (Scott and Ashes, 1993). Likewise, feeding protected canola supplements to lactating dairy cows led to a significant increase in C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 in milk (Ashes *et al.* 1992). To date, formaldehyde treatment is the only documented protection method allowing a large increase in the proportion of absorbed polyunsaturated fatty acids. Other forms of encapsulation, for example those that involve encapsulation using calcium alginate have not been very efficient (Ekeren *et al.* 1992).

1.6.2 Saponification (Calcium soaps)

Jenkins and Palmquist (1984) proposed this technique, because calcium soaps were insoluble in the rumen (Figure 1.11b), but dissociated in the acid environment of the abomasum. The preparation involved adding calcium chloride to molten sodium salts of the lipid. Calcium salts precipitated and the soap was air dried, before milling and in diets.

Tallow fatty acids depressed fibre digestibility from 51 % in control fed cows to 45 %, while the tallow calcium soaps gave a normal fibre digestibility of 50 %(Jenkins and Palmiquist, 1984). Elmeddah *et al.* (1991) did not observe any negative effects of calcium soaps based on palm oil. Sukhija and Palmquist, (1991) attributed this effects to the low dissociation of calcium salts at a normal rumen pH (6 to 7). However, recent work has reported extensive biohydrogenation of calcium salts, especially those of unsaturated fatty acids at a low rumen pH (less than 6.0). This has been attributed to the rapid decline in rumen pH upon feeding

calcium soaps (Ferlay *et al.* 1993; Enjalbert *et al.* 1994 and Wu *et al.*, 1991). The effect of rumen pH on calcium soaps has subsequently been confirmed by *in vitro* studies (Van Nevel and Demeyer, 1996 and Sukhija and Palmquist, 1990). Ferlay and Doreau, (1993) suggested that the absence of negative effects of calcium salts on rumen carbohydrate digestion, may be due to either a reformation of calcium salts after biohydrogenation, which were more stable, or to a positive action of ionised calcium in enhancing bacterial adhesion to feed particles.

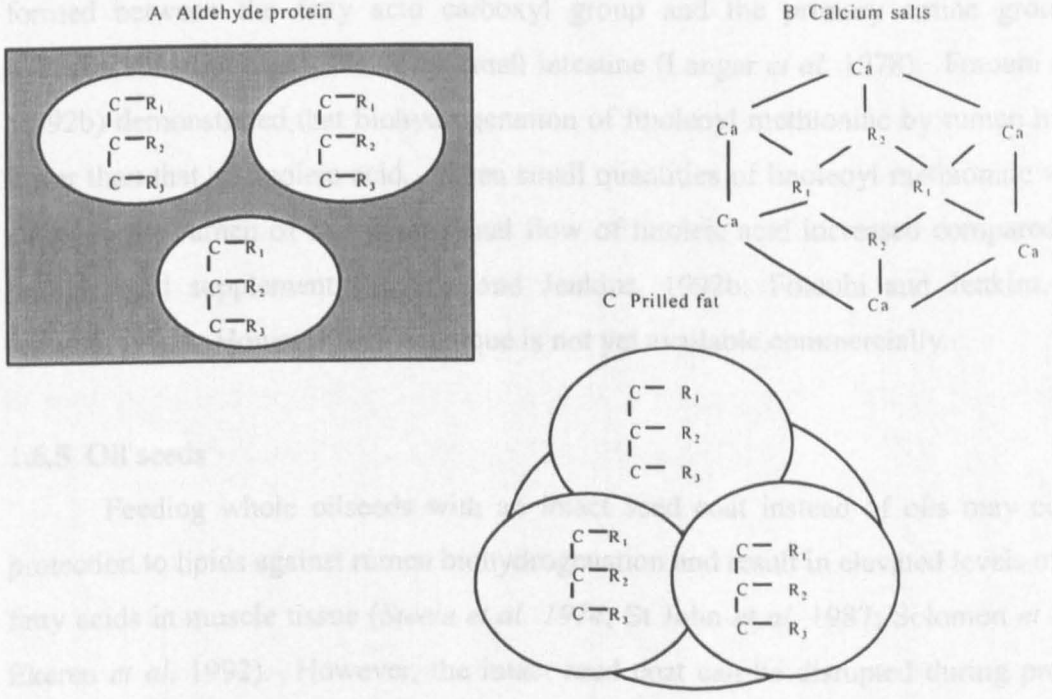


Figure 1.11 Techniques for protecting dietary fat

(Adapted from Scott and Ashes, 1993)

1.6.3 Prilled fatty acids

Prilling is the process whereby fatty acids are crystallized together in a matrix through a rapid cooling process which results in small spherical beads (Schauff and Clark, 1989; Figure 1.11c). Prilled fat supplements are inert in the rumen and do not affect nutrient digestion when supplemented at 3.5 % or less in the diet. Grummer, (1988), compared a relatively saturated fat source (48.6 %, C16:0; 35.1 %, C18:0; and 12.8 %, C18:1) fed as prilled fat to calcium salts of palm oil fatty acids. Even when hydrolysed saturated fatty acids react more readily with metal ions to form insoluble salts in the rumen when the rumen pH is

maintained above 6.0 (Palmquist, 1984; Palmquist and Conrad, 1978). Prilled fats based on C16 and C18 fatty acids (Schauff and Clark, 1989) or hydrogenated fish oils rich in C20 and C22 fatty acids (Doreau, 1992) have low to moderate inhibitory effects on rumen function.

1.6.4 Fatty acyl amides

Fatty acids readily react with amines to form fatty acyl amides that resist microbial breakdown in the rumen (Fotouhi and Jenkins, 1992a and Jenkins, 1995). The amide bond formed between the fatty acid carboxyl group and the primary amine group is rumen undegradable, but digestible in the small intestine (Langar *et al.* 1978). Fotouhi and Jenkins, (1992b) demonstrated that biohydrogenation of linoleoyl methionine by rumen microbes was lower than that of linoleic acid. When small quantities of linoleoyl methionine were directly added to the rumen of sheep, duodenal flow of linoleic acid increased compared with a free linoleic acid supplement (Fotouhi and Jenkins, 1992b; Fotouhi and Jenkins, 1992b and Jenkins, 1995). However this technique is not yet available commercially.

1.6.5 Oil seeds

Feeding whole oilseeds with an intact seed coat instead of oils may confer natural protection to lipids against rumen biohydrogenation and result in elevated levels of unsaturated fatty acids in muscle tissue (*Steele et al. 1974*; St John *et al.* 1987; Solomon *et al.* 1991 and Ekeren *et al.* 1992). However, the intact seed coat can be disrupted during processing and mastication, thus reducing the effectiveness of the technique. It is generally accepted that for cattle and ewes, grains or oilseeds should be coarse ground or rolled before feeding for efficient post ruminal digestion. This processing would consequently disrupt the seed coat and expose the lipids to microbial transformation upon ingestion. But because sheep efficiently masticate their feed there is no advantage in processing oil seeds (Ørskov *et al.* 1974).

1.7 DUODENAL LIPIDS

Duodenal lipids consist of hydrogenated dietary fatty acids (60 %), microbial fatty acids (35 %) and dietary fatty acid escaping microbial biohydrogenation in the rumen (5 %) (Moore and Christie, 1984). Transformed fatty acids include saturated fatty acids and *trans* positional isomers such as vaccenic acid, as well as branched and odd chain fatty acids from microbial lipids.

Sutton *et al.* (1970) demonstrated that in sheep on a high fibre diet, the total fatty acids entering the duodenum may exceed the amount ingested by upto 40 %, whilst by feeding high concentrate diets, this difference increased to 104 %. Additional evidence of the significant contribution of *de novo* microbial lipid synthesis to the duodenal fatty acid flow was provided by an observed increase microbial phospholipids at the duodenum. However, although the amount of C18:0 was in excess of that which could arise from ingestion and biohydrogenation, there was no accompanying increase in C16:0 concentration, which constitutes a higher concentration of microbial lipids than C18:0 (Noble, 1978).

These findings were attributed to several factors including, the underestimation of duodenal flow associated with sampling or application of digesta markers (Murphy *et al.* 1987). The absorption and degradation of the shorter chain fatty acids by the rumen epithelial cells (Jesse *et al.* 1992) or contamination by desquamation of epithelial cells, salivary and gastric secretions. In spite of these discrepancies, the consensus of opinion is that where the duodenal fat flow is in excess of that ingested in the diet then microbial synthesis is responsible (Wu *et al.* 1991). However, such is the influence of changes in the chemical and physical properties of the diet upon *de novo* microbial lipid synthesis, that this contribution is extremely variable as summarised in the review by Jenkins (1993).

Studies examining the absorption of long chain fatty acids from the rumen have shown that losses are negligible. For example, Wood *et al.* (1963) observed that 85 to 95 % of the ^{14}C18:2*n*-6 added to the rumen of sheep (with a ligated reticulo-omasal orifice) was recovered from the ruminal contents after 48 hours. Likewise the radioactivity (0.3 % of total count) was minimal in blood plasma of goats given a ruminal dose of labelled ^{14}C18:3*n*-3 and ^{14}C18:2*n*-6, indicating that fatty acids were not absorbed in significant amounts from the rumen, omasum

or abmosum (Bickerstaffe *et al.* 1972). Similarly, degradation of long chain fatty acids to carbon dioxide and volatile fatty acids was less than 1 % when fatty acids were incubated with ruminal microbes *in vitro* (Wu and Palmquist, 1991). Noble (1981) suggested that ruminal catabolism of fatty acids was low because fatty acid catabolism requires aerobic conditions.

Fatty acid losses from the rumen have however been reported while feeding diets with added fat to dairy cattle (Jenkins and Palmquist, 1986 and Wu *et al.* 1991) and to sheep (Sutton *et al.* 1975). In a review of the literature, Jenkins (1993) suggested that lipid losses across the rumen were 60.5 and minus 17.8 g/day in cattle, and 5.5 and 1.9 g/day in sheep offered control and diets with added fat, respectively. Microbial lipid synthesised 15 g/kg lipid free organic matter digested in the rumen was estimated from the linear regression equation relating duodenal lipid flow to lipid intake. This is similar to the value (14.4 g/kg fat free diet) estimated by Wu *et al.* (1991), in dairy cattle. The regression of duodenal lipid flow against dietary lipid intake gave a slope of 0.92, indicating losses of dietary lipids in the rumen averaging 8 g /100 g lipids intake. Higher losses (30 and 32 g/100 g of lipid intake, respectively) have been reported by Wu *et al.* (1991) and Bauchart *et al.* (1987) in dairy cattle.

Different fatty acids also disappear to different extents depending upon chain length. The highest disappearance of over 90 % was reported with fatty acids of shorter chain length (less than C14), whilst the lowest losses were observed in total C16 and C18 fatty acids (Wu *et al.* 1991). In studies by Jesse *et al.* (1992), C16:0 was oxidised and converted to ketone bodies by the rumen epithelial cells isolated from sheep. This breakdown of long chain fatty acids and absorption of shorter chain end products may explain the differences in losses across the rumen observed between long chain and shorter chain fatty acids. Using published data from different authors, Doreau and Ferlay (1994), reported a relationship (Figure 1.12) between duodenal flow of fatty acids and fatty acid intake represented in the equation below.

Doreau and Ferlay (1994), observed that most negative lipid balances at the duodenum occurred with high fat diets and diets based on fresh grass. Using this equation predictions can be made that dietary fat level below 5 % will result in positive fat balances across the rumen, whilst dietary fat levels above 5 % will give negative balances of fat across the rumen.

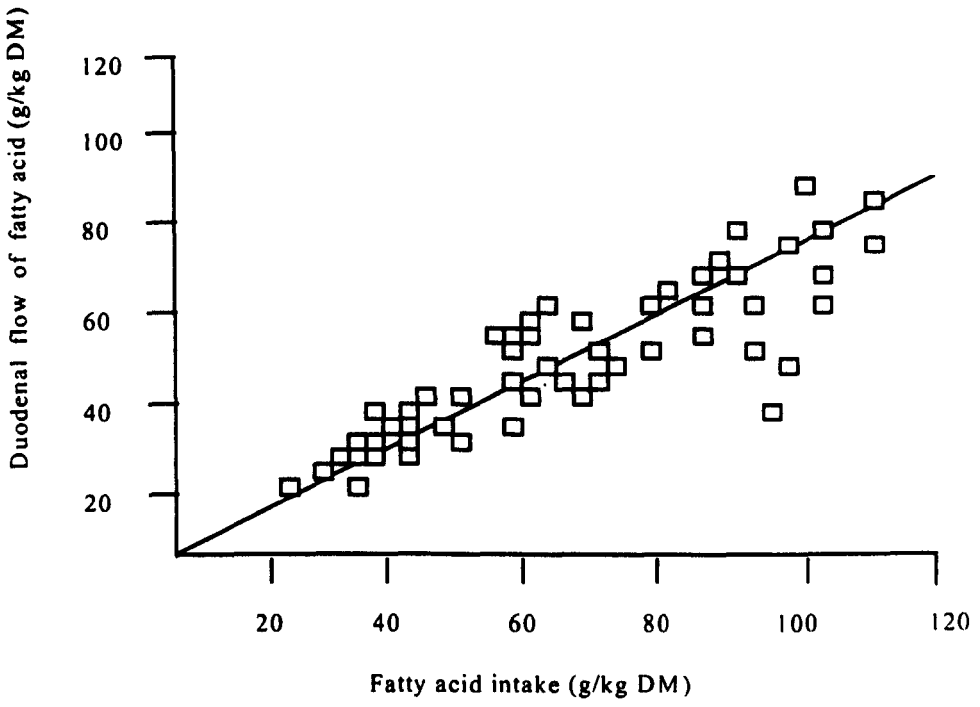


Figure 1.12 Relationship between duodenal fatty acid flow and fatty acid intake in ruminants (Data from Doreau and Ferlay, 1994.)

FAD = 0.801 FAI + 9.29 (n = 113, r = 0.87; RSD = 3.3 g/kg DM)

FAD = Fatty acid flow at the Duodenum

FAI = Fatty acid intake (g/kg DM intake)

1.8 POST RUMINAL DIGESTION, ABSORPTION AND TRANSPORT OF LIPIDS

Almost 90 % of dietary lipids reaching the duodenum are unesterified saturated fatty acids adsorbed onto particulate matter (Scott *et al.* 1969), except for microbial lipids. Triacylglycerols are also present in ruminant animals offered diets supplemented with protected fats or oils. Noble (1981), Moore and Christie (1984); Gurr and Harwood (1991) have extensively reviewed the digestion, absorption and transport of lipids in ruminants: Bauchart (1993).

1.8.1 Lipid digestion in the small intestines

The pancreatic duct in ruminant animals joins the bile duct 5-10 cm from the point of entry of the common duct into the duodenum and the flow rate of the biliary secretion is greater than the pancreatic secretion (Moore and Christie, 1984). The total lipid concentration of bile is 1400 mg/100 ml which consists of phosphatidylcholine (PC) (80 %), lysophosphatidylcholine (6.3 %), phosphatidylethanolamine (PE) (2.7 %), cholesterol (4.7 %) and cholesterol esters (2.%) (Lennox *et al.* 1968). The molar percentages of the principal fatty acids present in the PC are C16:0, 36 %; C18:0, 9.8 %; C18:1*n*-9, 27.9 %; C18:2*n*-6, 6.6 %; C18:3*n*-3, 4.9 % and intermediates or by products of rumen biohydrogenation (Christie, 1973). The concentration of bile acids is variable and the principal acids are taurcholate, glycocholate and other minor bile acids. Dietary fatty acids (30-40 g daily) entering the ovine duodenum are thus augmented with biliary lipids (10-15 g), which alter the lipid composition of the digesta by increasing the proportion of phospholipid, especially PC (Moore and Christie, 1984).

Sheep pancreatic juice contains two phospholipases. Phospholipase A_1 and A_2 are inhibited by acidic conditions of the duodenum (pH 2-3.5) and proximal jejunum (pH 3.6-4.2). Optimum activity for both enzymes is pH 5.6 and hence phospholipid hydrolysis begins at the mid-jejunum (pH 4.7-6.0) and continues throughout the distal jejunum (pH 6-7.6) (Moore and Christie, 1984). Phospholipase A_1 hydrolyses fatty acids at the *sn*-1 position of the phosphatidylcholine or phosphatidylethanolamine and releases mainly saturated fatty acids, whilst phospholipase A_2 hydrolyses fatty acids at the *sn*-2 position releasing the unsaturated fatty acids (Moore and Christie, 1984). There is also evidence that sheep pancreatic will hydrolyse the remaining ester linkage in the resulting lysophosholipids (Dawson *et al.* 1982).

Rumen protected lipids increase the amounts of triacylglycerols entering the duodenum. Under such circumstances, the digestion mechanism and solubilization of the liberated fatty acids is similar to that observed in non-ruminant (Moore and Christie, 1984). Triacylglyceride digestion takes place below the mid-jejunum and is catalysed by pancreatic lipase, which has an optimal activity at pH of 7.5 and 7.8, but in the presence of bile salts activity can occur below pH 5.0 (Arienti et *al.* 1974). For maximum lipase activity the presence of the polypeptide cofactor, colipase is essential (Julien *et al.* 1972). Pancreatic lipase catalyses the breaking of ester bonds in position *sn*-1 and *sn*-3 of triacylglyceride molecules and releases 2 moles of free fatty acids and 1 mole of 2-monoacylglycerides. These products are resistant to further hydrolysis and have an important role in micellar solubilisation of fatty acids (Thomson and Dietschy, 1981).

In spite of these changes in composition, the major proportion of digesta lipid remains associated with particulate matter (Moore and Christie, 1984). For example in the jejunal of sheep, 70 % of the total PC, 60 % of the lysophosphatiylcholine, and 78 % of the total unesterified fatty acids are adsorbed on to particulate matter (Leat and Harrison, 1969). Before absorption can occur the adsorbed lipids from exogenous and endogenous origins must be transferred to the soluble micellar phase, which is the function of the biliary constituents (Noble, 1978). Harrison and Leat, (1972) observed that lipid absorption was reduced in the absence of pancreatic juice, but were completely eliminated in the absence of bile. When sheep bile was added to duodenal contents *in vitro*, there was a transfer of unesterified fatty acids from the particulate matter into micellar solution (Scott and Lough, 1976). This increased with increasing pH and was later shown to be due to the combined action of bile salts and phospholipids (Smith and Lough, 1976). Above pH 4.0, PE and PC were equally effective in enhancing the solubilization of fatty acids by bile salts, but at pH 3.0 and below PE was more effective than PC. Ruminant bile is characterised by an excess of taurine over glycine conjugated bile acids, which aid micellar solubilisation (Moore and Christie, 1984). In the proximal jejunum of sheep, 60 % of the bile salts were partitioned in the soluble micellar phase and 40 % in the particulate phase (Harrisson and Leat, 1972).

1.8.2 Lipid absorption and intracellular metabolism

Ruminants absorb fats with a high degree of efficiency (80-90 %), as demonstrated with a variety of fats and fatty acids (Andrew and Lewis, 1970 a and b). In general, the ability

of ruminants to absorb C16 and C18 fatty acids is greater than that observed in non-ruminants (Noble, 1981). Lough and Smith, (1976), suggested that this difference between species may be due to the greater degree of dispersion of long chain saturated fatty acids in ruminant intestinal contents, and also the greater solubilization of saturated fatty acids by bile-salt/lysophosphatidylcholine micelles than by bile-salt/2-monoacylglycerides micelles.

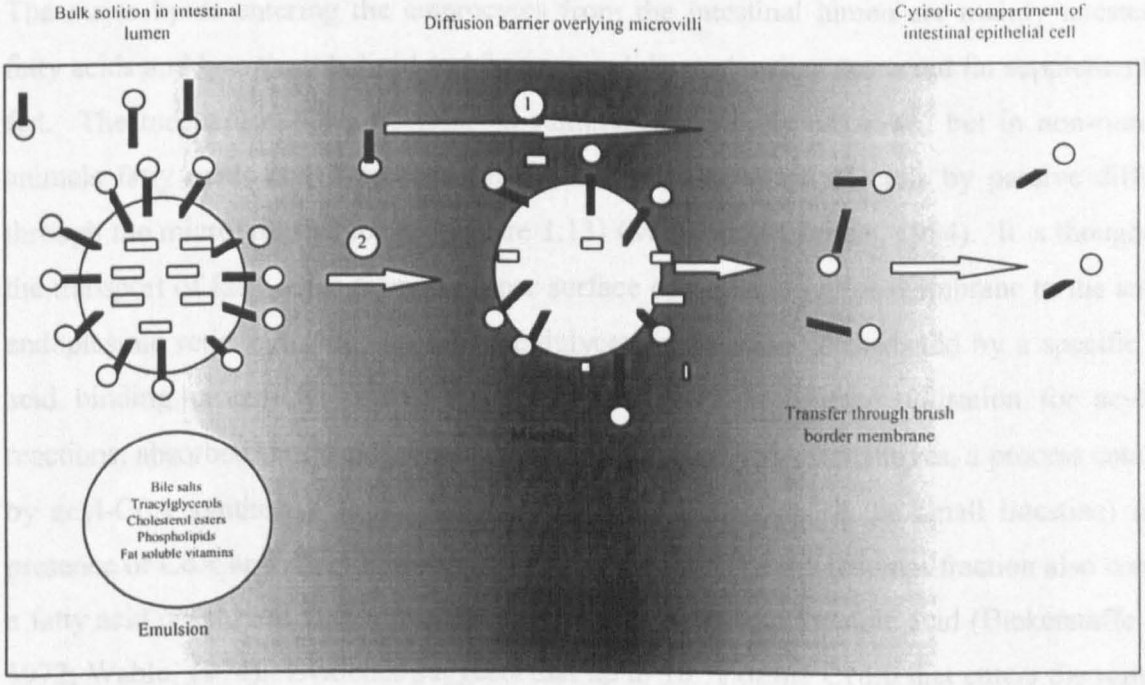


Figure 1.13 Diagrammatic representation of the lipid absorption (Modified from Gurr and Harwood, 1996).

Effects of biliary secretions in overcoming the diffusion barrier resistance offered by the unstirred water layer. In the absence of biliary secretions, individual molecules must diffuse across the barrier overlying the microvillus border of the intestinal epithelial cells (arrow 1). Hence uptake of molecules is diffusion limited. In the presence of biliary secretions (arrow 2), large amounts of these lipids are delivered directly to the aqueous membrane interface so that the rate of uptake is greatly enhanced.

The brush border membrane of the enterocyte is separated from the bulk fluid phase in the intestinal lumen by the unstirred fluid layer that is poorly mixed with the bulk phase (Figure 1.13). In ruminants, mixed micellar mainly consist of non-esterified fatty acids, lysophospholipids and bile salts (Moore and Christie, 1984). Unsaturated fatty acids and monoacylglycerides require a lower bile acid concentration for micellar solubilisation than

saturated fatty acids and their monoacylglycerides, whilst triacylglycerides are not incorporated into micelles in significant amounts, and this property is probably responsible for their low rate of absorption (Bauchart, 1993). The presence of bile salts in micelles helps to incorporate very insoluble non-polar molecules like cholesterol and fat soluble vitamins into micelles which subsequently aids absorption (Brindley, 1984).

The major lipids entering the enterocytes from the intestinal lumen are mainly unesterified fatty acids and lysophospholipid and 2-monoacylglycerols when protected fat supplements are fed. The mechanism for absorption in ruminant animals is unknown, but in non-ruminant animals fatty acids and 2-monoacylglycerols enter the intestinal cells by passive diffusion through the microvilli membrane (Figure 1.13) (Moore and Christie, 1984). It is thought that the transport of fatty acids from the inner surface of the microvillus membrane to the smooth endoplasmic reticulum, the site for triacylglyceride synthesis is mediated by a specific fatty acid binding protein (Z protein) (Ockner *et al.* 1972). Before utilisation for acylation reactions, absorbed fatty acids must be converted to their CoA derivatives, a process catalysed by acyl-CoA synthetase (located in the microsomal fraction of the small intestine) in the presence of CoA and ATP (Gurr and Harwood, 1996). The microsomal fraction also contains a fatty acid desaturase that converts C18:0 into *cis*-9,10-octadecenoic acid (Bickerstaffe *et al.* 1972; Wahle, 1974). Evidence suggests that up to 10 % of the C18:0 that enters the ruminant intestinal mucosa is desaturated to C18:1 before it appears in the lymph.

The synthesis of triacylglycerols in the ruminant enterocyte can occur by the α-glycerolphosphate pathway or the monoacylglycerol pathway, depending on the substrate availability (Figure 1.14). However, the principal pathway involves α-glycerophosphate, phosphatidic acid and diacylglycerols as intermediates (Cunningham and Leat, 1969) but ruminants can also synthesise triacylglycerols using the monoacylglycerol pathway, when fed diets containing protected fat supplements. The synthesis of phospholipid in the mucosal cells of the small intestines in ruminants occurs by the acylation of 1-lysophospholipids absorbed from the intestinal lumen (Figure 1.14) (Leat and Harrison, 1974). The mucosal cells actively synthesise cholesterol, a major component of lipoproteins that is rarely present in ruminant diets (Scott and Cook, 1975; Figure 1.14).

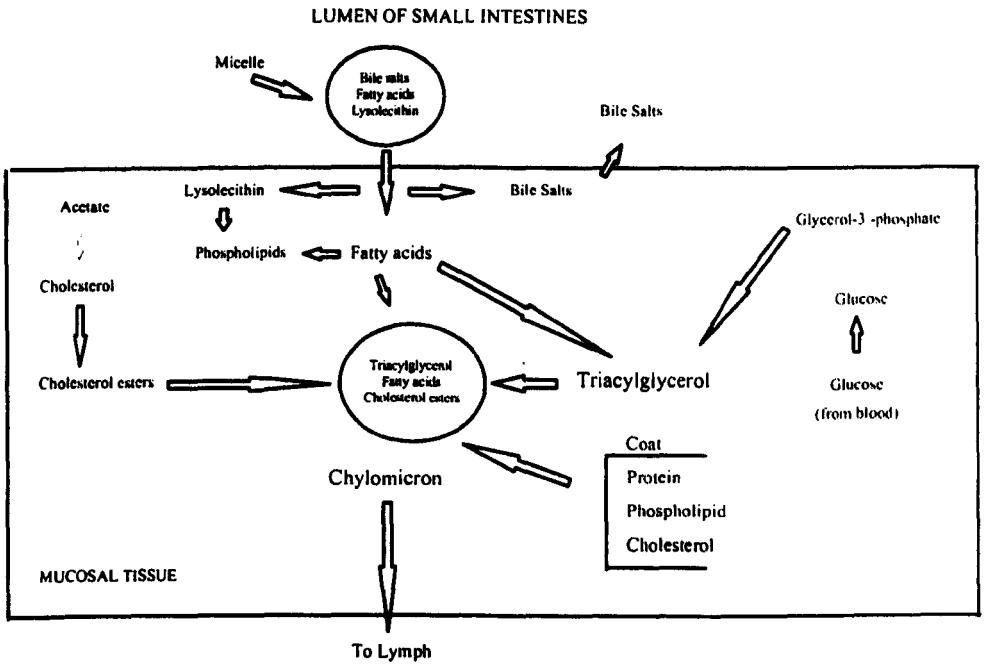


Figure 1.14 Metabolism of lipids in the enterocyte of the small intestines

(Adapted from Moore and Christie, 1984)

Different fatty acids are selectively incorporated into the various lipid classes synthesised in the mucosal cells of the ruminant intestines, and this selectivity is reflected in the fatty acid composition of the lymph lipids (Moore and Christie, 1984). For example in the intestinal lymph of sheep given a normal diet (forage), the triacylglycerols fraction contained 7 % of the polyunsaturated fatty acids (C18:2*n*-6 and C18:3*n*-3), compared to 27 % in the PC and 24.5 % in the cholesterol esters and was attributed to the rapid turnover of the triacylglycerol fraction (Christie, 1981). A higher proportion of saturated fatty acids was observed in the triacylglycerols and cholesterol esters (Christie and Hunter, 1978). However, when large amounts of polyunsaturated fatty acids (PUFA) are absorbed from the ruminant small intestines, PUFA maybe incorporated in the lymph triacylglycerols (Harrison *et al.* 1974).

1.8.3 Whole tract lipid digestibility

Whole tract lipid digestibility (measured between mouth and rectum), often suggests that fatty acid digestibility increases with increased concentration in the diet (Andrews and Lewis, 1970a and b; Sharma *et al.* 1978). Such results have been criticised based on the variable ruminal fatty acid balance. It is thus not surprising that digestibility coefficients in

such studies are often positive when animals are offered diets with no added fat and negative when diets are supplemented with fat.

In a review of the literature, (Doreau and Ferlay, 1994), the intestinal lipid digestibility (measured between the proximal duodenum and terminal ileum) showed a wide variation ranging between 52 to 92 %. Dietary fatty acid composition did not correlate with fatty acid digestibility both within and between experiments, for example, Soya oil fatty acid digestibility was 70 % and 92 % in studies by Hagemeister and Kaufmann, (1979) and Borsting *et al.* (1992), respectively. Methodological errors alone cannot conclusively explain these variations, since different values have been reported in the same experiments. Doreau and Chilliard, (1997), hypothesised that micelle formation varies between fatty acids or bile production depends on the nature of fat. Bauchart, (1993) suggested that the availability of fatty acids at the duodenum for absorption maybe limited, due to the association of fatty acids with cellular feed and microbial structures.

Individual fatty acid digestibility are reported to be higher in dairy cows offered diets containing calcium soaps of palm fatty acids compared to those offered diets containing animal-vegetable blends (Wu *et al.* 1991). This effect was attributed to the stability of calcium soaps in the rumen (at pH greater than 6.0) which in turn increased the proportion of unsaturated fatty acid flow to the small intestines. Sklan *et al.* (1985) observed that unsaturated fatty acids were more digestible than saturated ones of the same length due to their hydrophilic nature, which aided micelle formation and consequently increased absorption. However, the relative differences in efficiencies of absorption of individual fatty acids are far smaller in ruminants, because the majority of the unsaturated fatty acids are biohydrogenated in the rumen.

Noble *et al.* (1978), suggested that the absorption efficiency of medium and long chain fatty acids (C14 to C18), increased with the introduction of double bonds or with increased chain length (C18:1*n*-9 > C16:0 > C18:0). In contrast, Doreau and Ferlay (1994), observed that lipid digestibility depended on chain length, but did not significantly differ between fatty acids of C16 and C18 chain lengths (79 and 77 %, respectively). Wu *et al.* (1991), however observed a lower C16:0 digestibility compared to C18:0, which they associated with the high content of C16 in bile secretion. This increases the endogenous contributions, which in turn

reduced the apparent digestibility. Other factors associated with the low C16:0 digestibility are hind gut biohydrogenation consequently low apparent digestibility of saturated fatty acids. Similarly, hind gut microbial fatty acid synthesis increases faecal fatty acids output especially C16:0, when organic matter digestion is shifted to the hind gut with fat supplementation (Demeyer, 1991; Ferlay *et al.*, 1992). From the literature, it appears that the ability of animals to absorb a particular fatty acids is affected by many factors, including the physical and chemical properties of the non lipid components of the diet. This is probably why digestibility results from different trials are frequently contradictory and cannot be readily correlated.

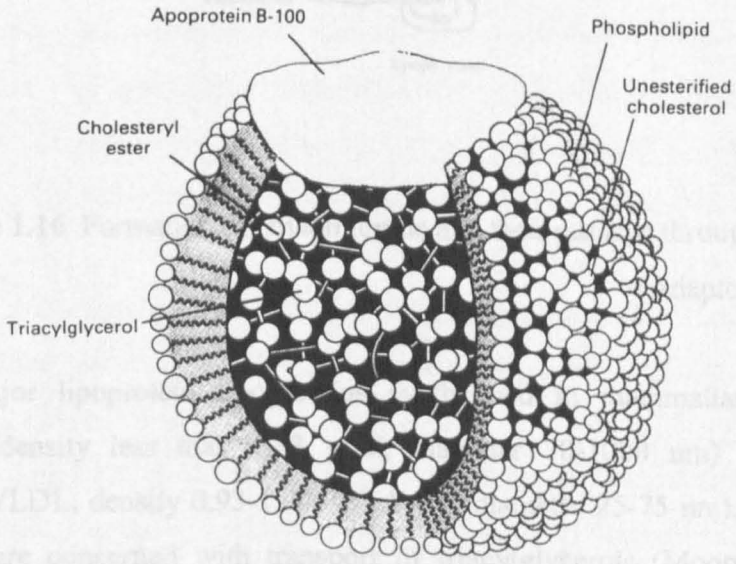


Figure 1.15 A model structure of the low-density lipoprotein

(Adapted from Gurr and Harwood, 1994)

1.8.4 The synthesis of lipoprotein and their secretion into lymph

Following absorption, the lipid components (triacylglycerols, phospholipid, cholesterol and cholesterol esters) and a number of apoprotein synthesised in the enterocyte are assembled into lipoprotein particles (Moore and Christie, 1984). Apolipoproteins confer specificity on the lipoprotein and determine the way in which they are metabolised and largely the metabolism tissues (Figure 1.15).

Newly assembled lipoprotein particles accumulate in the golgi apparatus in the supra nuclear region of the enterocyte (Gurr and Harwood, 1996). Migration of the Golgi vesicles to the basolateral region of the cell is followed by discharge of the lipoprotein particles into the intercellular space by reverse pinocytosis involving fusion of the Golgi membrane with

basolateral cell membrane (Figure 1.16) (Brindley, 1984). The lipoprotein then pass into the lacteals, and into the blood system via the intestinal and thoracic lymph ducts (Sterzing *et al.* 1971).

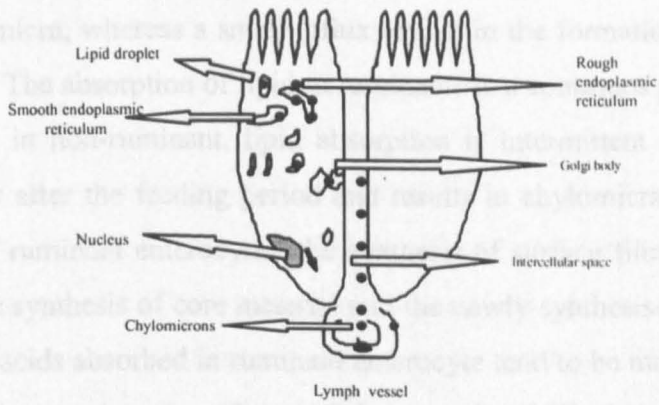


Figure 1.16 Formation of chylomicrons and their passage through the enterocyte

(Adapted from Brindley, 1984)

The two major lipoprotein lipoproteins synthesised in mammalian enterocytes are the chylomicra (density less than 0.93 g/ml, diameter 70-1000 nm) and very low-density lipoprotein (VLDL, density 0.93-1.006 g/ml, and diameter 25-75 nm). These two classes of lipoproteins are concerned with transport of triacylglycerols (Moore and Christie, 1984). However, in the lymph of sheep, there is a continuo spectrum of particle size from chylomicron to VLDL (Gooden *et al.* 1979), and attempts to distinguish between the two maybe artificial. Chylomicron-like particles from bovine plasma are composed of triacylglycerols, 87 %; phospholipid, 4 %; cholesterol, 4 %; cholesterol esters, 2 %; protein, 3 %. Similarly, bovine VLDL are composed of triacylglycerols, 74 %; phospholipid, 7 %; cholesterol, 7 %; cholesterol esters, 5 %; protein 8 % (Ferrein and Elbein, 1982). Chylomicron and VLDL phospholipid consist mainly of phosphatidycholine with smaller proportions of sphingomyelin and phosphatidylethanolamine (Christie and Hunter, 1978; Ferrein and Elbein, 1982). Chylomicra and VLDL particles consist of an inner core containing triacylglycerols and cholesterol esters, about 30 % of the free cholesterol, whilst the surface film consists of phospholipid, apoprotein and free cholesterol which together form a monolayer with a constant thickness of about 2.2 nm (Smith *et al.* 1978).

In ruminants, absorbed fatty acids are preferentially incorporated into the VLDL triacylglycerides and as a result the phospholipid to triacylglycerol ratio is greater than that in non-ruminants (Leat and Harrison, 1974). This species difference is partly because of two reasons. First, a large influx of triacylglycerol through the intestinal cells results in the formation of chylomicra, whereas a small influx results in the formation of VLDL (Redgrave and Dunne, 1975). The absorption of lipid in ruminants is a continuos process that operates at low rates, whereas in non-ruminant, lipid absorption is intermittent and is relatively rapid during immediately after the feeding period and results in chylomicra synthesis (Moore and Christie, 1984). In ruminant enterocytes, the synthesis of surface film components normally keeps pace with the synthesis of core material and the newly synthesised lipoprotein (VLDL). Secondly, the fatty acids absorbed in ruminant enterocyte tend to be more saturated than those in non-ruminants. In experiments with rats, Ockner *et al.* (1969), found that the absorption of saturated fatty acids resulted in the formation of VLDL, whilst the absorption of equivalent amounts of unsaturated fatty acids resulted in the formation of chylomicra.

In sheep fed a control diet, 72.6 % of the lymph lipids were transported in VLDL and only 27.4 % as chylomicra, the total lymph contained 18 % phospholipid and the major fatty acid in the lymph triacylglycerols were C18:0 and C16:0 (61.6 %) and C18:1 (14.9 %). After infusing maize oil (52.5 %, C18:2*n*-6) into the duodenum, there was a three fold increase in the rate of flow of lymph fatty acids and 61.5 % of the lymph was transported as chylomicra and 38.5 % as VLDL (Harrison *et al.* 1974). It is not known whether ruminant lymph contains lipoproteins other than chylomicron and VLDL, but rat mesenteric lymph contains small amounts of low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

1.8.5 Metabolism of triacylglycerol-rich lipoprotein in the blood

The metabolism of chylomicra and intestinal VLDL after entry into the blood stream is similar in ruminants and non-ruminants (Palmiquist, 1976). On entering the plasma, chylomicra and VLDL acquire apo-E and apo-C by transfer from plasma high-density lipoprotein (HDL) synthesised in the liver (Blum, 1980). This acquisition of additional apo-C ensures that the catabolism of chylomicra and VLDL is diverted from the liver towards the extra hepatic tissues. All the apo-C components (I, II and III) inhibit the removal of chylomicra and VLDL by the liver, whilst apo-C_{II} activates the enzyme lipoprotein lipase which is bound to the endothelial surfaces of the capillaries that permeate skeletal muscle, fat

depots and the mammary glands (Moore and Christie, 1984). The core triacylglycerols of plasma chylomicra and VLDL, fortified with apo-C_{II}, are hydrolysed by lipoprotein lipase to fatty acids and partial acylglycerol. These are taken up at the site of hydrolysis and utilised for energy production, the synthesis of adipose tissue triacylglycerol or the synthesis of milk fat (Moore and Christie, 1981; Vernon, 1981).

The hydrolysis of chylomicra and VLDL results in the removal of 80 % of the core triacylglycerol, and the conversion of these lipoproteins to smaller remnants particles or intermediate-density lipoprotein (IDL), which contain most of the core cholesterol esters. The conversion also results in the loss of surface components apo-C, apo-A_I, apo-A_{VI}, cholesterol and phospholipid which are transferred to high density lipoprotein (HDL) circulating in the plasma (Moore and Christie, 1984). Evidence from experiments with rats suggests that some of the surface phospholipid may be hydrolysed by extra hepatic phospholipases and the products of hydrolysis taken up by tissues (Eisenberg and Levy, 1975). If this process occurs in ruminant animals, it might provide a means whereby small amounts of essential fatty acids (virtually absent from ruminant plasma triacylglycerols and unesterified fatty acids) are distributed to the various tissues of the body (Moore and Christie, 1984).

In man the remaining core triacylglycerols in IDL are hydrolysed by hepatic lipase and the products taken up by the hepatocyte (but not the apo-B). The remaining particle is known as the low-density lipoprotein (LDL) and cholesterol ester account for 80 % of the core lipid. LDLs are the major transport vehicles for cholesterol in the blood. Specific high affinity receptors on the plasma membrane of target cells interact with the apo B on the LDL surface and fuse with lysosomes for further metabolism (Bauchart, 1993). Skeletal, intestinal and hepatic tissues located in plasma membrane regions known as pits produce the LDL receptors.

Many aspects of lipoprotein metabolism in ruminants remain to be resolved. It is however clear that polyunsaturated fatty acids are directed to lipid classes other than the triacylglycerols in the plasma lipoproteins, with the consequence that lipid storage tissues in the animal are relatively deficient in these components (Moore and Christie, 1984).

1.9 FATTY ACID SYNTHESIS

The adipose tissue is the principal site for synthesis of fatty acids *de novo* from acetate and the major site for desaturation of C18:0 to C18:1*n*-9 in ruminants animals (Vernon, 1981). Fatty acids are derived from endogenous synthesis from low molecular weight precursors such as glucose and acetate and from exogenous origin mainly the diet or synthesis in other tissues and transported via the plasma lipids.

1.9.1 Anabolism (lipogenesis)

In ruminating animals, acetate is the most important precursor whereas glucose and possibly lactate may make a more substantial contribution in non-ruminants (Vernon *et al.* 1981). The limited utilization of glucose results from the virtual absence of two key enzymes of the citrate-cleavage pathway (ATP-citrate lyase and NADP-malate dehydrogenase). The source of the reducing equivalents necessary for fatty acid synthesis in ruminant's adipose tissue is the pentose-phosphate cycle and the isocitrate cycle (Bauman and Davis, 1975). The utilisation of acetate as a carbon source for fatty acid synthesis and the generation of the NADPH via the isocitrate cycle represents a distinct advantage to the ruminant animal in sparing glucose (Bauman and Davis, 1975).

Fatty acid synthesis *de novo* requires a source of cytosolic acetyl CoA and NADPH. The cytosolic acetyl-CoA is carboxylated to form malonyl-CoA by carboxoxylase, the enzyme that provides the regulatory step to control fatty acid synthesis (Stryer, 1988). Malonyl-CoA is the carbon donor, which provides two carbon units for sequential condensation by fatty acids synthetases to form long chain fatty acids. Palmitic acid (C16:0) and stearic (C18:0) are the principal fatty acids synthesised *de novo* in proportions governed by acetate concentration and by whether exogenous unesterified fatty acids are present (Deeth and Christie, 1979).

Compared to non-ruminants, ruminants have a low rate of fatty acid synthesis which has been attributed to restrictions at several steps in the pathway (Vernon, 1981). Robertson *et al.* (1982) have demonstrated that this restriction is linked to the rate of glycolysis which in turn seems to be regulated by pyruvate kinase. A major control point at pyruvate rather than glycolysis, perhaps reflects the fact that glucose is the major source of glycerol-3-phosphate for esterification. Glucose oxidation via the pentose phosphate pathway, probably supplies the

majority of the NADPH required for fatty acid synthesis in the ruminant adipose tissue (Christie, 1981).

Studies have shown that sheep (Prior, 1978) and cattle (Prior and Jacobson, 1979) adipose tissue can use lactate as a substrate for fatty acid synthesis. Lactate can be incorporated into the fatty acids at a faster rate than acetate (Prior and Jacobson, 1979). Although not conclusive lactate is converted to fatty acids via the citrate cleavage pathway and hence the inability of glucose to act as a substrate for fatty acid synthesis may be due to the inhibition of Pentose phosphate pathway before pyruvate, and not a deficiency of ATP citrate lyase. Lactate maybe more important as a lipid precursor in *ad-libitum* fed than in restricted fed sheep (Prior, 1978) and also when the diet is based on silage or fermented feed.

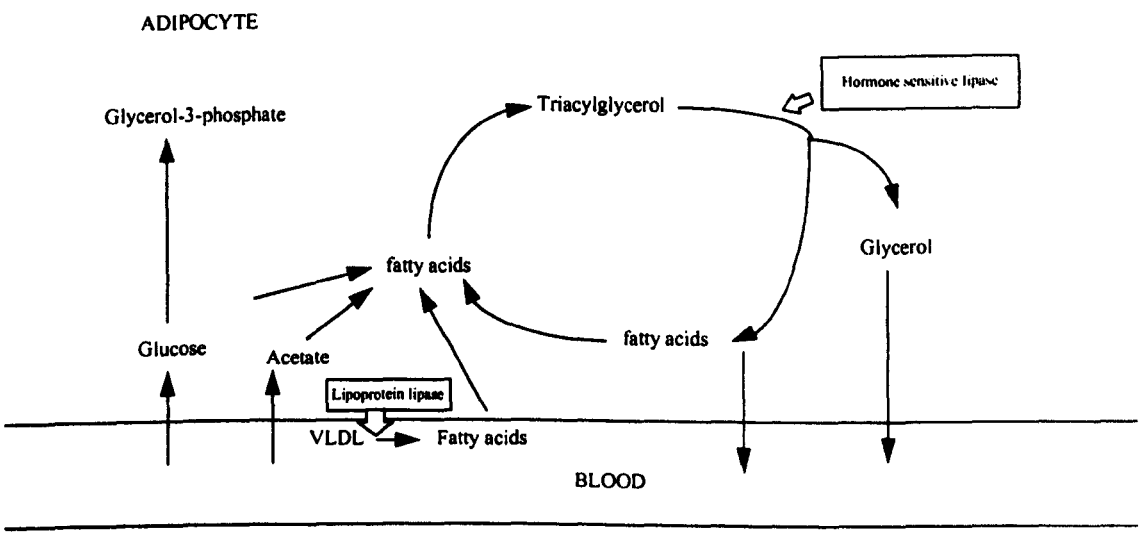


Figure 1.17 Pathway for the synthesis and hydrolysis of triacylglycerol

(Adapted from Vernon, 1981)

In addition to *de novo* fatty acid synthesis, fatty acids maybe acquired from the blood plasma via the lipoprotein lipase (LPL) (Figure 1.17). LPL is secreted by adipocytes and transported to endothelial cells lining capillaries where plasma triacylglycerides hydrolysis occurs prior to uptake (Nilsson-Ehle *et al.* 1976). Long chain free fatty acids can then transverse membranes and thus become available to the adipocyte for storage. In addition, plasma free fatty acids bound to albumin are available to the adipocyte. Tume and Thornton, (1985) demonstrated

that serum from fed sheep was more effective than serum from fed rats in activating sheep adipose tissue LPL at low substrate concentrations. But in the same study serum taken from both species on a restricted plane of nutrition, stimulated adipose tissue LPL activity at physiological substrate concentrations. This increased activity promoted by either species differences and plane of nutrition would ensure that those tissues (e.g. cardiac and skeletal muscle) which continue to synthesise LPL during fasting or nutritional restriction are able to assimilate the relatively low amounts of circulating triacylglycerol. Christie *et al.* (1986) observed that LPL had little specificity for the hydrolysis of particular fatty acid bonds in the primary positions of the triacylglycerol and phosphatidylcholine molecules. Thus the lipoprotein lipase mechanism is important in providing not only dietary fatty acids, the source of essential fatty acids as well as non-essential fatty acids, but also in redistributing lipoprotein fatty acids (Mersmann, 1991).

Triacylglycerol synthesis involves the esterification of long chain fatty acids to the three carbon precursor, glycerol-3-phosphate. Two fatty acids are initially esterified by one or two enzymes (glycerol phosphate acyltransferase) to form phosphatidic acid which is dephosphorylated by phosphatidate phosphohydrolase to yield a diacylglycerol that is finally esterified with a third fatty acids by diacylglycerol acyltransferase to yield triacylglyceride. This pathway has not been studied extensively in the ruminant adipose tissue. Triacylglycerol biosynthesis is stimulated by insulin and inhibited by adrenergic hormones in the rat adipose tissue *in vitro* (Saggerson, 1985).

1.9.2 Catabolism

Degradation or lipolysis is initiated by the hormone sensitive lipase and results in the production of free fatty acids and glycerol (Figure 1.18). The fatty acids may enter the adipocyte fatty acid pool and can be re-esterified or be transported to the exterior (Fain, 980). A cascade type regulatory system beginning with the activation of a membrane-bound hormone receptor which causes the dissociation and activation of a GTP-binding protein (Gs) which interacts with and activates adenylate cyclase controls lipolysis. Adenylate cyclase catalyses the synthesis of cyclic AMP which activates cyclic AMP-dependent kinase (A-kinase), which phosphorylates hormone sensitive lipase (Styer, 1988). Recent studies suggest that this chain of events also cause the translocation of hormone sensitive lipase from the cytosol to the fat droplet surface (Gurr and Harwood, 1996).

In ruminants catecholamines are potent stimulators of lipolysis whereas glucagon has only a slight effect (Vernon, 1981). Both glucagon and catecholamines exert their effects through the same pathway, by interacting with a specific receptor on the plasma membrane. Changes in response and sensitivity to catecholamines and adenosine occur during various physiological states e.g. during pregnancy. Factors and mechanisms responsible for these responses have not been fully elucidated in farm animals, but growth hormone, thyroid hormone, glucocorticoids, sex steroids and even insulin maybe involved (Vernon, 1981).

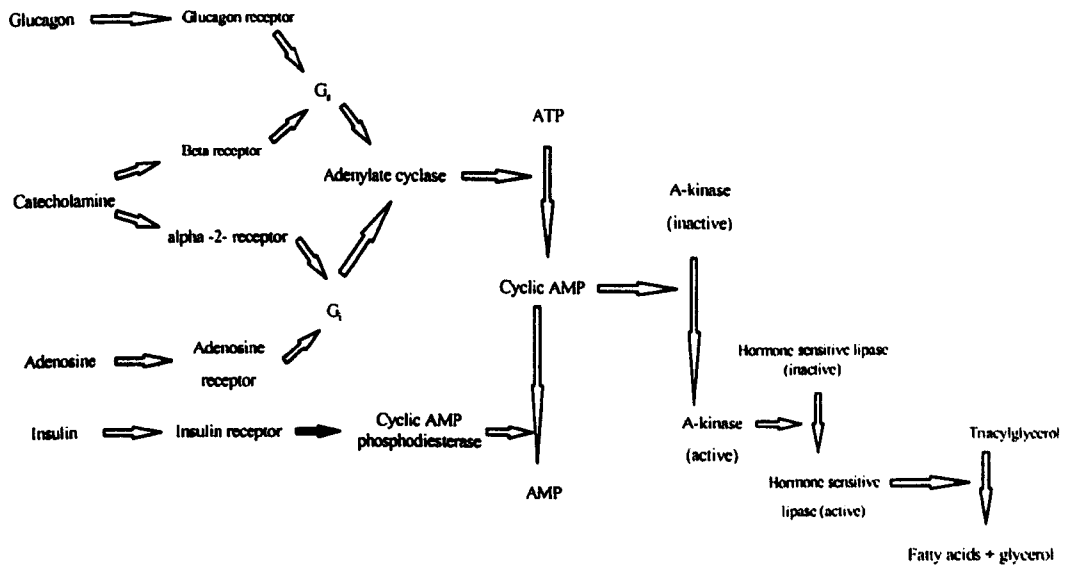


Figure 1.18 Lipolytic signal transduction cascade

1.9.3 Fatty acid elongation and desaturation in tissues

Since palmitic acid (C16:0) is the major product of fatty acid synthetase, other mechanisms are required to produce longer or shorter chain fatty acids. Two elongation pathways exist which extend the chain by two carbon units; one in the mitochondria, which uses acetyl-CoA and NADH or NADPH for reduction and one in the microsomes, which uses malonyl CoA and NADPH but differs from fatty acid synthetase which is a cytosolic enzyme (Enser, 1984). Fatty acids also undergo shortening by sequential removal of two carbon units. Fatty acid desaturation is also important in maintaining fluidity in the adipose tissue and phospholipid membranes as well as in converting the major dietary essential fatty acids (C18:2*n*-6 and C18:3*n*-3) into more unsaturated longer chain derivatives which are required for normal growth and maintenance (Gurr and Harwood, 1996). For example ingested C18:2*n*-6 is converted C18:3*n*-6 (γ-linolenic acid), C20:3*n*-6 (eicosa-8,11,14-trienoic),

C20:4*n*-6 (docosa-7,10,13,16-tetraenoic) and C22:5*n*-6 (docosa-4,7,10,13,16-pentaenoic) acids by alternating sequences of desaturation and elongation steps (Figure 1.5). The same desaturase and elongase enzymes also desaturate and elongate C18:1*n*-9 and C16:1*n*-7, thus evoking a corresponding fatty acid series. From these series only C18:2*n*-6 and C18:3*n*-3 produce essential fatty acid families while C18:1*n*-9 and C16:1*n*-7 families can be totally synthesised by the animal (Sprecher, 1981).

According to Gurr and Harwood, (1996) fatty acid desaturation follows certain rules:

1. All desaturases require molecular oxygen and a reduced pyridine nucleotide to catalyse the desaturation of preformed fatty acids in the form of CoA esters.
2. For a given enzyme the double bond is always introduced into the methylene chain at a fixed position from the carboxyl group e.g. Δ9 desaturase always introduces a double bond between carbons 9 and 10, and not 8 and 9 or 10 and 11.
3. When the substrate is a saturated fatty acid, the first double bond is inserted between carbons 9 and 10. Unlike plants, animals cannot insert double bonds farther away than 9 carbons from the carboxyl group. e.g. C16:0-Δ9-C16:1 and C18:0-Δ9-C18:1.
4. When the substrate is already unsaturated, subsequent double bonds are inserted between the double bond nearest the carboxyl group in such a way to maintain the methylene interrupted distribution of double bonds e.g.

$$CH_3\,(CH_2)_7CH{=}CH(CH_2)_7\,COSCoA \quad \Delta 9\text{-oleoyl-CoA}$$

$$\downarrow$$

$$CH_3(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4COSCoA \quad \Delta 9,6\text{-Linoleyl-CoA}$$

5. In a metabolic pathway leading to the formation of a PUFA, desaturation usually alternates with elongation

Desaturation activity is present in sheep intestinal mucosa but absent form the intestines of pigs and chicken (Bickerstaffe and Annison, 1972) thus indicating the necessity for ruminants to modify the saturated fatty acids produced by rumen biohydrogenation of dietary lipids. The desaturase of sheep in the adipose tissue was also higher than in the liver but in rats and chicken the liver activity was greater (Wahle, 1974).

The activities of Δ9, Δ6 and Δ5 desaturase are highly dependent on nutrition and hormonal control (Sprecher, 1981). Due to the key position of Δ6 desaturase in the biosynthetic pattern of the essential and non-essential PUFA series, the regulation of its activity plays a role in the production of all highly unsaturated acids (Brenner, 1989). Several mechanisms acting either directly on the enzyme activity or through the induction of enzyme biosynthesis control the activity of Δ6 desaturase. For example, Δ6 desaturase enzyme desaturates different substrates at different speeds and this competition between the natural substrates C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3, results in the absence of C18:1*n*-9 desaturation and elongation products, in animals offered diets containing high levels of the other two fatty acids. However, because of the extensive loss of essential fatty acids in the rumen, the products of C18:1*n*-9 metabolism are higher than in other meat animals (Enser, 1984).

1.10 MUSCLE LIPIDS

The intramuscular lipids consist of triacylglycerols present in the intramuscular adipose tissue, the marbling fat and as microscopic droplets between muscle fibres (Enser and Wood, 1997). The concentration of the marbling fat increases as the carcass weight and fatness increases and can vary from less than 1 % to over 30 % (Wood, 1990)

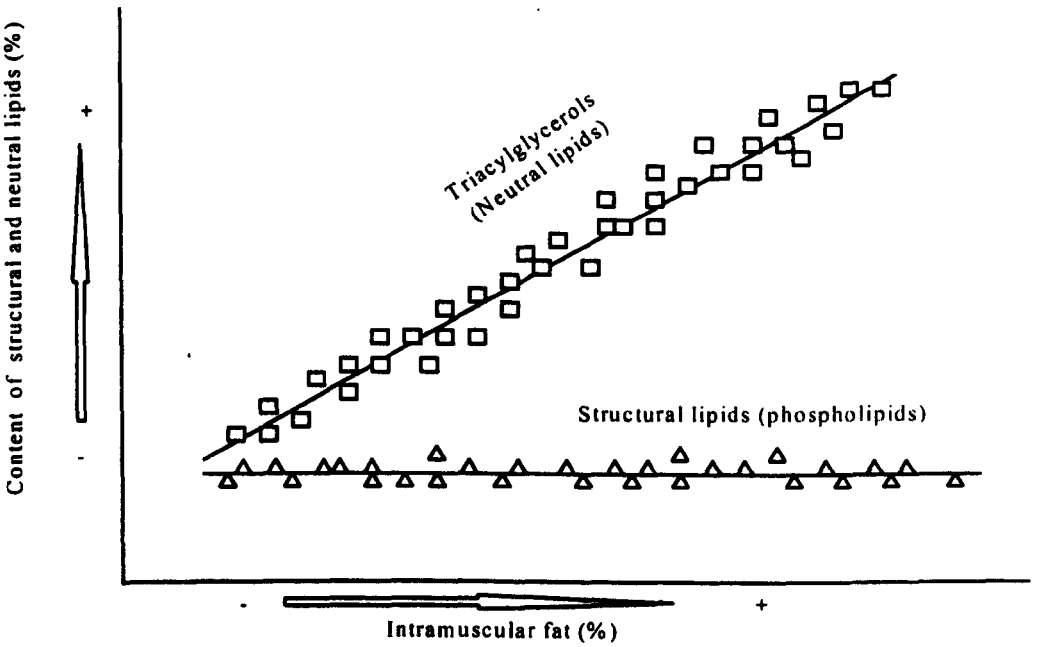


Figure 1.19 Distribution of structural and neutral lipids in intramuscular lipids

(Adapted from Opstvedt, 1984)

Structural lipids consist mainly of phospholipid and comprise on average 0.75 % of the muscle weight (Enser and Wood, 1997). Phospholipid have a slower rate of growth than triacylglycerol or total lipids and all classes increase more rapidly in the fat depots in the muscle (Figure 1.19). Whereas the fatty acid composition of the triacylglycerols resembles that of the adipose tissue, phospholipid contain high proportions of polyunsaturated fatty acid (Marmer *et al.* 1984). Therefore, unlike the adipose tissue of ruminants, intramuscular lipids contain measurable quantities of C20 and C22 polyunsaturated fatty acids (Enser *et al.* 1996).

1.10.1 Membrane lipids

Singer and Nicholson (1972) proposed a fluid mosaic model for the membrane structure (Figure 1.20). In the model an asymmetric phospholipid bilayer forms the basis of the membrane structure with proteins spanning the membrane or embedded into the hydrophobic core region. Biological membranes are not constant in composition, but are continually being remodelled to meet specific requirements, in relation to transport of nutrients, involvement in cell signalling pathways or providing an appropriate micro environment for the function of membrane protein/enzymes (Yeagle, 1989).

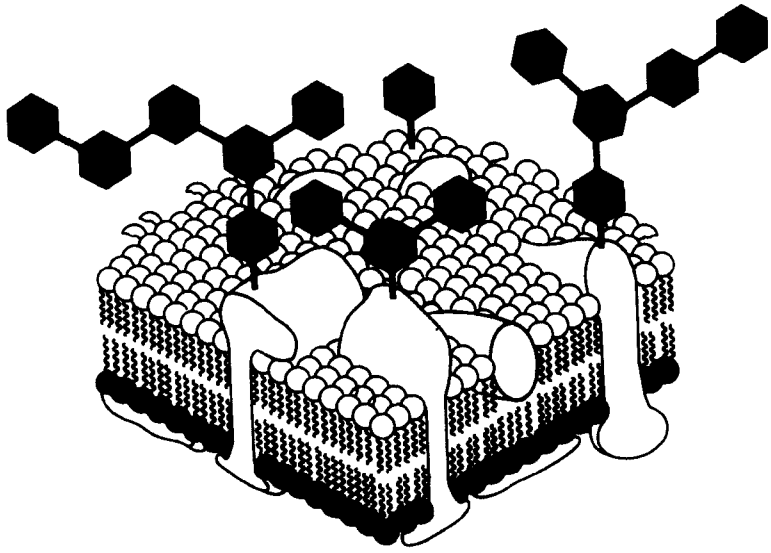
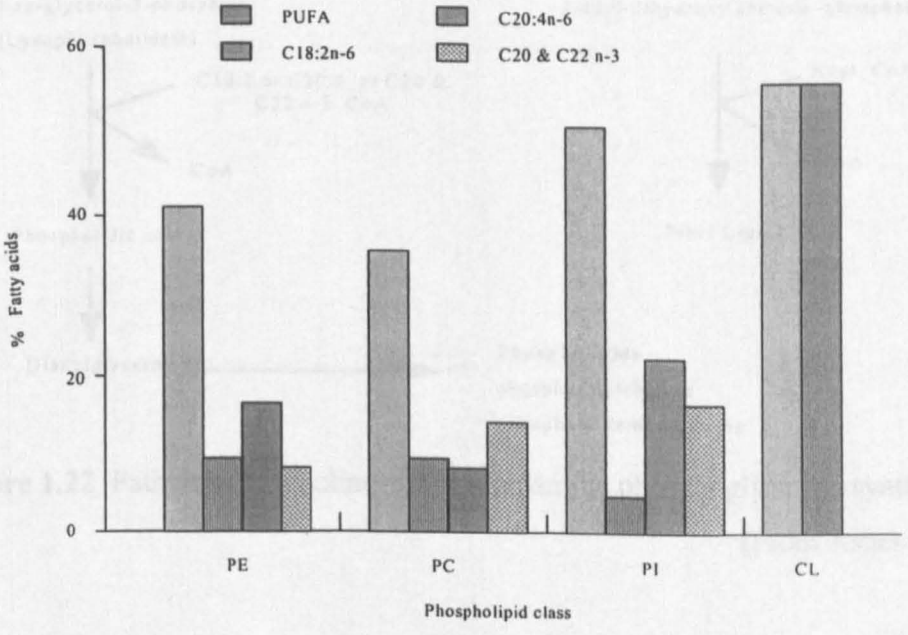


Figure 1.20 The Singer and Nicholson fluid-mosaic proposal

(Adapted from Gurr and Harwood, 1996)

The phospholipid fraction of membranes consists of phosphatidylcholine (PC; 50-60 %) and phosphatidylethanolamine (PE; 20-35 %) (Gandemer, 1997), and relatively small proportions of cardiolipin and phosphadidylinositol (PI) are 2-9 % and 3-9 %, respectively. Muscle phospholipids also contain small amounts of sphingomyelin and phosphotidylserine (together 2 %). Phospholipid content and composition are related to the metabolic type of muscle. Glycolytic muscles are mainly composed of glycolytic fibres using carbohydrates as a fuel and contain small amounts of mitochondria and myoglobin and are thus referred to as 'white muscles' (Turkii and Campbell, 1967). Oxidative muscles are mainly composed of oxidative fibres using fatty acid as fuel and are rich in mitochondria and myoglobin ('red muscles') and thus contain more phospholipid than glycolytic muscles (Marmer *et al.* 1984).

The fatty acid composition of phospholipid is characterised by high levels of linoleic acid (C18:2*n*-6; 14 -30 %) and trace amounts of long chain polyunsaturated fatty acids (PUFA) such as arachidonic acid (C20:4*n*-6; 8-14 %), C20:5*n*-3, C22:4*n*-6, C22:5*n*-3 and C22:6*n*-3 (Gandemer *et al.* 1997). Phospholipids are membrane components and large variations in fatty acid composition would alter membrane properties (Yeagle, 1989). In contrast, large differences in fatty acid composition have been observed between phospholipid classes (Christie, 1984). Thus both the proportion and type of PUFA are completely different from one phospholipid class to the other. For example, a typical fatty acid composition of the main phospholipid classes of cattle liver is shown in Figure 1.21. Cardiolipin and PE are the most unsaturated classes, although C18:2*n*-6 is high in cardiolipin whilst PE contain a large amount of longer chain PUFA. PC contains PUFA formed by a large amount of C18:2*n*-6 and a low proportion of C20:4*n*-6 (Christie, 1978).



PE, phosphatidylethanolamine; PC, phosphatidycholine; PI, phosphatidylinositol; CL, cardiolipin

Figure 1.21 PUFA composition of individual phospholipid classes from liver lipids of cattle (Data from Christie, 1981)

The biosynthesis of phospholipid in mammalian cells is summarised in Figure 1.22. The first step involves the acylation of either glycerol-3-phosphate (P) or dihydroxyacetone-P to form phosphatidic acid, which is dephosphorylated to produces diacylglycerol (Scott and Ashes, 1993). The diacylglycerol, in the presence of the appropriate phosphotransferase, combines with CPD-choline or CPD-ethanolamine to produce phosphatidylcholine (PC) or phosphatidylethanolamine (PE) (Tijburg *et al.* 1989). During the biosynthesis, C16:0 and C18:0 acids are esterified in the *sn*-1 position while unsaturated fatty acids (C18:1, C18:2*n*-6, C20:4*n*-6) are generally found in position *sn*-2 of the glycerol molecule.

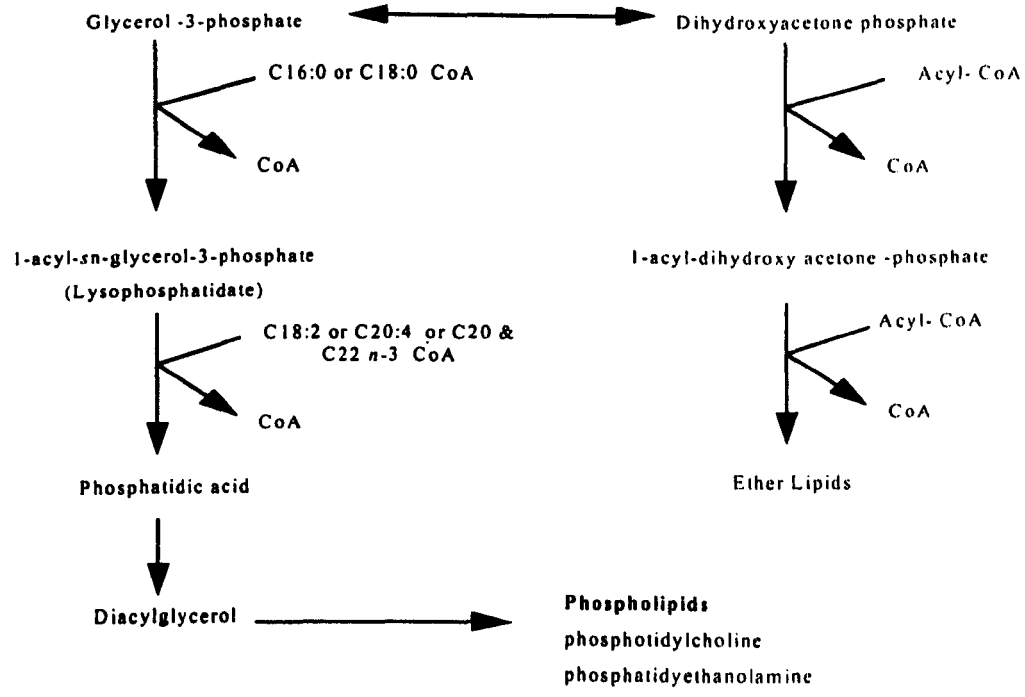


Figure 1.22 Pathways for choline and ethanolamine phosphoglyceride synthesis

(From Ashes *et al.* 1993)

The inclusion of protected fats containing different fatty acid composition in ruminant diet results in substantial changes in the molecular species profile of the choline phospholipids in muscle. Large increases in the proportions of C16:0/C18:0, C18:0/C18:2*n*-6 and a reduction in C16:0/C18:1*n*-9, was reported while feeding diets containing protected rapeseed oil containing 60 %, C18:1*n*-9; 20 %, C18:2*n*-6 and 10 %, C18:3*n*-3. This demonstrates that although the ratio of C18:1 to C18:2*n*-6 in the diet was 3:1, C18:2*n*-6 was preferentially incorporated into the phospholipid fraction (Ashes and Scott, 1993). Marmer *et al.* (1984)

reported a preferential partitioning of C18:2*n*-6 into the phospholipid (polar) fraction compared to C18:3*n*-3 (Table 1.7). Protected fish oil (17 % C20:5*n*-3) in the diet increased the proportion of C16:0/C20:5*n*-3 and reduced the proportion of C16:0/C18:1n-9 in the phosphatidylcholine fraction (Ashes *et al.* 1992b).

Table 1.7 *Fatty acid composition of neutral and polar lipids fraction as a function of tissue site*

	Semitendinosus	Psoas major	Longissimus
Neutral lipid			
C18:2*n*-6 (mg/)100 g)	55	87	70
C18:3*n*-3 (mg/100 g)	6	11	6
C18:2*n*-6 (%)	33	36	43
C18:3*n*-3 (%)	55	11	66
Polar lipids (phospholipids)			
C18:2*n*-6 (mg/100 g)	110	153	94
C18:3*n*-3 (mg/100 g)	5	5	3
C18:2*n*-6 (%)	66	64	57
C18:3*n*-3 (%)	45	31	33

(From Marmer *et al.* 1984)

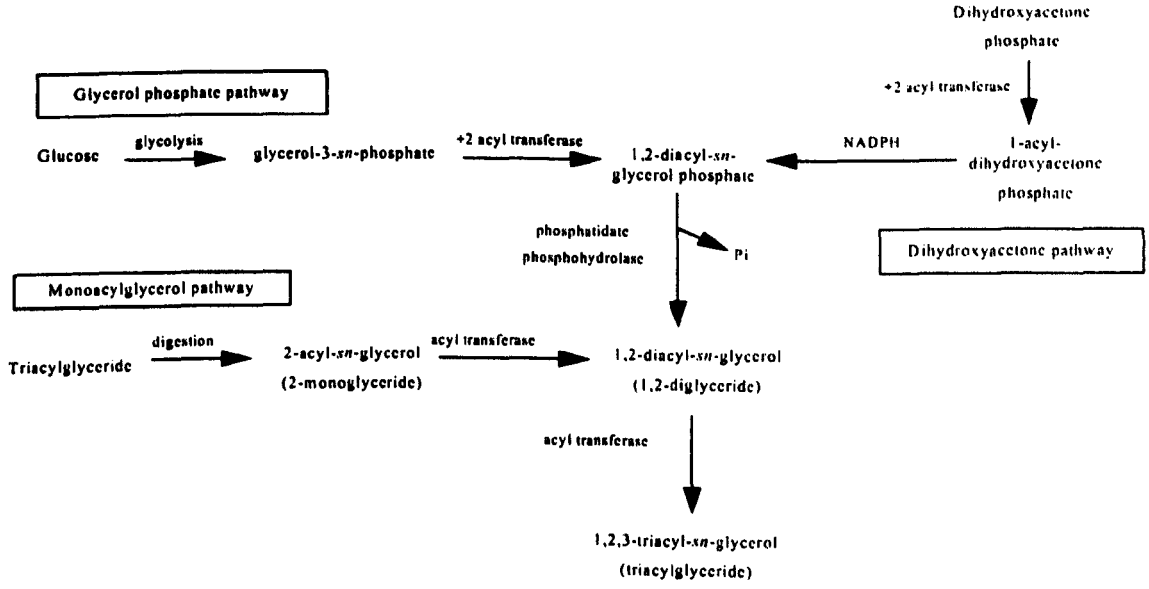


Figure 1.23 Biosynthesis of triacylglycerol

(Adapted from Enser, 1984)

1.10.2 Adipose tissue lipids

Triacylglycerol synthesis in the adipose tissue may follow three pathways (Figure 1.23). The glycerol phosphate pathway, a product of glycolysis pathway provides the glycerol backbone of the triacylglycerols, which links phospholipid and triacylglycerol metabolism (Enser, 1984). In the dihydroxyacetone phosphate pathway, glycerol is derived from dihyroxyacetone rather than the glycerol phosphate (Gurr and Harwwod, 1996). Evidence suggests that this pathway is reduced in conditions of starvation or when the animal is on a high fat diet. Monoacyglycerols from lipid digestion, may also act as substrates for triacylglycerol synthesis in the monoacylglycerol pathway. Specific fatty acids from endogenous or exogenous sources maybe introduced at the acylation stages.

1.10.3 Growth of the adipose tissue

During postnatal growth, nutrients are partitioned largely between muscle and fat. Adipose tissue can increase through either hyperplasia (increase in cell number) or hypertrophy (increase in cell size) the latter being entirely due to lipid accretion. In lambs there is an apparent rapid increase in cell number over the first hundred days of age in subcutaneous and intermuscular depots, followed by a quiescent period after which there is a further burst of hyperplasia (Vernon, 1986). Smith *et al.* (1987) showed that these changes were paralleled by changes in acetyl CoA carboxylase activity. In general fattening is associated with increased lipogenic flux, esterification and also lipoprotein lipase activity. The rate of lipogenesis changes during growth and varies with anatomical site (omental > perirenal > subcutaneous > intermuscular > intramuscular), breed and physiological state of the animal (Hood, 1982). Lipogenic activity increased in the adipose tissue of Holstein steers during weight gain (Pothoven and Bietz, 1973). During fasting and consequent weight loss, there was a decline in the capacity of the adipose tissue to synthesise fatty acids but this capacity was restored after re-alimentation (Hood and Thornton, 1980). Dietary energy increased lipogenic activity of large and small-frame steers (Scott and Prior, 1980). Lipogenesis was also greater in small frame steers (470 kg), when compared to large frame steers (Hood and Allen, 1979), whilst small frame steers were also fatter than large frame steers at a common carcass weight or age.

Hood and Thornton (1980) observed that adipocytes deposited lipids to a maximum cell diameter of about greater than 280 μm, after which the rates of synthesis and hydrolysis are at

equilibrium. This is in agreement with earlier findings that lipogenic enzyme activity in bovine adipose tissue increased cell volume until a critical cell volume. Hood, (1982) suggested that a limiting amount of water per cell controls this mechanism. Water, either bound or free, must be present to facilitate the action of cytoplasmic enzymes utilising water soluble substrates. Thus enhanced lipogenesis after periods of starvation (Hood and Thornton, 1980), maybe due partly to an increase in the intracellular cytoplasmic space.

1.11 DIETARY EFFECTS ON FATTY ACID COMPOSITION IN MEAT

The rumen has a major effect on the composition of tissue lipids in that it is the site of hydrogenation of a high proportion of dietary unsaturated fatty acid. Ruminant fat tissue thus differs from that of non-ruminants, in containing a higher proportion of saturated fat and a lower proportion of polyunsaturated fatty acids (PUFA) (Table 1.8).

In the muscle of retail samples of beef sirloin steak, lambs chops and pork chops purchased from UK supermarkets (Table 1.8), total fatty acids were found to be 3.8 ± 1.3 %, 4.9 ± 1.7 % and 2.3 ± 0.3 % of tissue weight respectively (Enser *et al.* 1996). However, the P:S ratio was higher in pork than in either beef or lamb, although the *n*-6:*n*-3 ratio was beneficially lower in beef and lamb than in pork, reflecting dietary lipid composition as well as species variations. It is however worthy noting that fatty acid ratios are not always important when the absolute values are small. There are many reported examples of modifications in tissue fatty acid composition via the diet, all of which involve manipulation of rumen fermentation patterns as discussed below.

Table 1.8 *Fatty acid composition of the loin muscle (mg/g) in steaks or chops purchased from four supermarkets*

Fatty acid	Beef	Lamb	Pork
C12:0 lauric	2.9	13.8	2.6
C14:0 myristic	103	155	30
C16:0 palmitic	962	1101	526
C18:0 stearic	507	898	278
C18:1 *trans*	104	231	-
C18:1*n*-9 oleic	1395	1625	759
C18:2*n*-6 linoleic	89	125	302
C18:3*n*-3 α-linolenic	26	66	21
C20:3*n*-6	7	2	7
C20:4*n*-6 arachidonic	22	29	46
C20:5*n*-3 eicosapentaenoic	10	21	6
C22:5*n*-3 docosapentaenoic	16	24	13
C22:6*n*-3 docosahexaenoic	2	7	8
Total fat	3835	4934	2255
P:S	0.11	0.15	0.58
n-6:*n*-3	2.11	1.32	7.22

P:S = polyunsaturated to saturated fatty acid ratio (C18:2*n*-6 + C18:3*n*-3)/ (C12:0+C14:0+C16:0);

(From Enser *et al.* (1996)

1.11.1 Forage and grain diets

Ørskov *et al.* (1974) demonstrated that sheep fed whole barley produced firmer fat than those fed on rolled or processed barley due to lower concentrations of C7 and C18

branched chain fatty acids. Branched chain fatty acids were synthesised from propionic acid whose production increased when the soluble carbohydrate content of the diet was increased.

The fatty acid composition of concentrate (grain-based) and forage (grass-based) diets are different and lead to different fatty acid compositions in tissues. Enser *et al.* (1998) observed that grazed (grass) lambs had a fat composition similar to that of steers finished on grass. Compared to young bulls given barley-soybean concentrate diets, the steers had 3.1 times more C18:3*n*-3 and increased levels of all other *n*-3 fatty acids, whilst the bulls had higher (2.5 times more than grass fed) concentrations of C18:2*n*-6 and all other *n*-6 fatty acids. Lamb muscle however contained higher levels of C18:0 and total PUFA, especially the *n*-3 PUFA than the steers. As a consequence the *n*-6 to *n*-3 ratio was lower in grass fed lambs (1.0) than in steers (1.3), while the ratio in cattle offered concentrate diets was 9.8. Similar results for lamb in comparison to beef (Table 1.8) were reported by Enser *et al.* (1996). Sumida *et al.* (1972) had earlier observed that cattle fed on pasture had higher levels of C18:0 and lower levels of unsaturated fatty acids than cattle fed in drylot, probably due to variations in rumen biohydrogenation.

Marmer *et al.* (1984) observed that beef cattle offered concentrate diets were considerably fatter than those offered grass diets. Total fatty acids in the *semitendinosus* muscle was 4105 and 1966 mg/100 g muscle tissue in the concentrate and grass fed cattle, respectively. As a result of these differences in fatness, the intramuscular concentration (%) of C18:2*n*-6, were similar in both concentrate (4.0 %) and grass (4.4 %) fed cattle, showing a production system effect, since a higher value would have been expected in grass fed because of the lower fatness. Hence, at low levels of fat, the contribution made by the phospholipids is proportionately greater than at higher fat levels. In contrast, the weights of C18:3*n*-3 and all *n*-3 PUFA per 100 g muscle were higher in grass fed animals despite the lower total fatty acid content (Marmer *et al.* 1984).

1.11.2 Supplemental fats

(a) Oils and Oilseeds

St John *et al.* (1987) reported that steers offered whole rape seed in conjunction with a high energy corn-based diet for 100 days had lower concentrations of C16:0 in both the lean

and adipose tissue lipids. Feeding steers diets supplemented with high oleate sunflower seed also increased the C18:1*n*-9 content of the adipose tissue (Ekeren *et al.* 1991). Similarly, Clinquart *et al.* (1991) observed increased concentrations of C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 and a reduction of C14:0, C16:0 and C18:0 in steers after feeding Soya oil or steam-flaked linseed in a concentrate ration. Overall, these observations confirm earlier suggestions that a limited amount of dietary unsaturated fatty acids (unprotected), for example C18:3*n*-3 in grass lipids and C18:2*n*-6 from concentrate diets, escapes rumen biohydrogenation are deposited into tissues lipids.

Scott *et al.* (1971), obtained a ten fold elevations of C18:2*n*-6 in the perirenal and subcutaneous adipose tissue of sheep by feeding supplements of safflower oil protected by formaldehyde-treated casein. Thornton and Hood, (1976), offered steers a protected safflower fat supplement until the subcutaneous fat contained 20 % C18:2*n*-6, and then returned them to a conventional diet and observed little change in the C18:2*n*-6 concentration over a period of 300 days. This suggests that changing the C18:2*n*-6 or other PUFA content of existing fat depots is a very slow process and can be best achieved by rapidly fattening lean, young animals on a diet with a high content of a protected PUFA supplement in this case C18:2*n*-6 fatty acid. This may also explain the wide variation in responses reported in the literature. For example Dinus *et al.* (1974), offered steers (470 kg live weight) diets containing 5.7 % protected safflower oil and observed a gain in body weight (BW) equivalent to 0.05 kg/100 kg BW/day and after 48 days feeding the C18:2*n*-6 content of the adipose tissue had increased from 2.9 % to 5.8 %. Faichney *et al.* (1972) offered young cattle (live weight 185 kg) diets containing 10 % protected safflower oil and reported a growth rate of 0.45 kg/100 kg BW/day. After 56 days the C18:2*n*-6 content of subcutaneous fat had increased to 23 % and of perirenal fat to 34 %. Thus, by protecting unsaturated fatty acids from rumen biohydrogenation the amount of tissue unsaturation can be increased to levels similar above those obtained in non-ruminant animals.

The impact of feeding protected fat sources on the fatty acid profile of adipose tissue from feedlot steers is presented in Figure 1.24. The inclusion of protected canola seeds (PCS) increased the proportion of C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 in the subcutaneous adipose tissue (Ashes *et al.* 1993), whilst feeding protected tallow resulted in little change in the fatty acid profile (Garret *et al.* 1976). The proportion of C18:2*n*-6 increased three fold from 1.5 to

4.5 % of the fatty acids and this was further increased to 5.3 % by feeding 15 % PCS in the ration with similar increases in the omental and perirenal adipose tissue. The proportion of C18:3*n*-3 in the different adipose tissues increased five fold from 0.3 % to 1.5 % as a result of feeding 10 % PCS, and this was increased to 2 % when 15 % PCS was included in the diets.

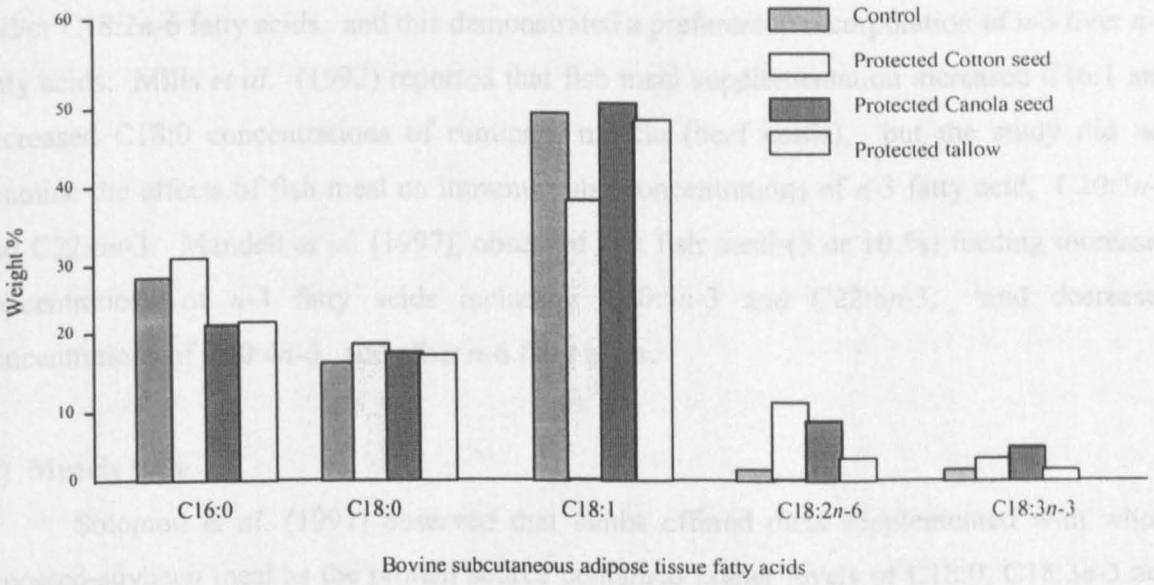


Figure 1.24 Effects of feeding different sources of formaldehyde treated oilseeds on the fatty acid profiles

(Adapted from Ashes *et al.* 1993)

In contrast, increases in the proportion of C18:1*n*-9, which accounts for approximately 60 % of the C18 fatty acids in canola oil (rapeseed), were much less (from 45.6 % to 48.0 % in the subcutaneous fat after feeding 10 % PCS in the diet) (Ashes *et al.* 1993). The failure to observe larger increases in C18:1*n*-9 may be due to the inhibition of the Δ9 desaturase enzyme which converts C18:0 to C18:1 in the adipose tissue (St John *et al.* 1991). The latter observation seems unlikely because there was no accumulation of the precursor acid, C18:0. A more probable explanation is that dietary C18:1*n*-9 from PCS was incorporated into the triacylglycerols fraction of the adipose tissue, thus reducing endogenous fatty acids synthesis. This would also explain the decreased in C16:0, which is the principle end-product of *de novo* fatty acid synthetase system (Ashes *et al.* 1993). The fatty acid profiles obtained in the adipose tissue of steers fed PCS indicates that C18:2*n*-6 and C18:3*n*-3 are preferentially incorporated into the triacylglycerols, and this results is similar to that obtained by Ashes *et al.* (1992a), for milk from dairy cows fed PCS.

(b) Fish oil

Ashes *et al.* (1992b), observed that the inclusion of protected fish oil in the diet of sheep, containing 17 % C20:5*n*-3, increased the proportion of C16:0/C20:5*n*-3 and reduced the proportion of C16:0/C18:1 in the phospholipids. The authors suggested that dietary C20:5*n*-3 and its metabolites in muscle phospholipids increased through substitution with C18:1*n*-9 and/or C18:2*n*-6 fatty acids, and this demonstrated a preferential incorporation of *n*-3 over *n*-6 fatty acids. Mills *et al.* (1992) reported that fish meal supplementation increased C16:1 and decreased C18:0 concentrations of ruminant muscle (beef cattle), but the study did not examine the effects of fish meal on intramuscular concentrations of *n*-3 fatty acid, C20:5*n*-3 and C22:6*n*-3. Mandell *et al.* (1997), observed that fish meal (5 or 10 %) feeding increased concentrations of *n*-3 fatty acids including C20:5*n*-3 and C22:6*n*-3, and decreased concentrations of C20:4*n*-6, and other *n*-6 fatty acids.

(c) Muscle type

Solomon *et al.* (1991) observed that lambs offered diets supplemented with whole rapeseed-soybean meal as the protein source contained higher levels of C18:0, C18:3*n*-3 and C20:4*n*-6 compared to lambs on soybean meal or rapeseed meal. Additionally, the *semimembranosus* and *triceps brachii* muscles from all treatments contained 12 to 19 % more PUFA than the *longissimus* muscle (Solomon *et al.* 1991). The latter observation is similar to that made by Enser *et al.* (1998), who reported that while *gluteobiceps* muscles were relatively high in total fatty acids, *longissimus* muscle had the least and tended to have the lowest PUFA concentrations, associated with its metabolic status as a 'whiter muscle'. Although the relative differences in C18:3*n*-3 content as a percentage of total fatty acids were small in the latter study, they illustrate these muscle differences in lamb: *triceps brachii* 2.3 %, *gluteobiceps* 2.3 % and *longissimus* 1.9 %.

Eichorn *et al.* (1985) observed that the *semitendinosus* muscle contained 6 % more PUFA than the *longissimus*, while the *triceps brachii* contained an intermediate value in both bulls and steers. The authors attributed this observation to marbling differences between the muscles, with increasing marbling resulting in increased amounts of saturated fatty acids. Enser *et al.* (1998), similarly observed that the *longissimus* muscle of cattle was lower in PUFA than the *triceps brachii* and *gluteobiceps*, for example C18:3*n*-3 as a percentage of total fatty acids was; *triceps brachii* 1.4; *gluteobiceps* 1.5 and *longissimus* 1.2. The fatty acid composition of the

subcutaneous adipose tissue was reported to be similar to that of muscle triacylglycerol fractions, suggesting that intramuscular fat (marbling) is similar in fatty acid composition to subcutaneous adipose tissue (Eichorn *et al.* 1985). The adipose tissue of ruminants animals is high in C18:1*n*-9 and C16:0 and low in C20 and C22 fatty acids due to the low proportion of phospholipid in the total lipid fraction (Enser *et al.* 1996), and the failure of ruminant adipose tissue to incorporated these fatty acids into the triacylglycerols (Storry *et al.* 1974), despite the fact that they escape rumen biohydrogenation (Ashes *et al.* 1992b).

(d) Sex

Higher levels of PUFA as a percentage of total fatty acids have been reported in bulls than in steers, although not all the C20 and C22 fatty acids were described (Hood and Allen, 1971 and Eichorn *et al.* 1985). These differences in fatty acids composition between the sexes were expressed based on the ratio of phospholipid to triacylglyceride, which is associated with differences in carcass fatness. In the study of Eichorn *et al.* (1985), bulls had two fold higher percentages of C18:2*n*-6 and C18:3*n*-3 in muscle fatty acids than steers, both of which were proportional to the higher ratio of phospholipid to triacylglycerols in bulls because they are leaner. However, in the study of Enser *et al.* (1998), the percentage C18:3*n*-3 was higher in fatter grass fed steers, emphasising the importance of production systems rather than differences observed between sexes

1.12 GENETIC EFFECTS ON FATTY ACID COMPOSITION

1.12.1 Fat content

Breed affects the composition and quality of fat tissue mainly through its effects on total fat content (Leat, 1977). At the same body weight, late maturing breeds have a lower concentration of fat than early maturing breeds, and the fat tissue itself has a higher concentration of water and a low concentration of lipids in smaller cells (Wood, 1984). Using the changes in composition that occurred as carcass weight increased from 15 to 21 kg in four sheep breeds, Wood *et al.* (1980) estimated the relative growth and maturing rates of tissue and fat depots (Table 1.9), using the growth coefficient *b* in the allometric relationship;

$$\text{Log}_{10} Y = a + b \log_{10} X$$

(Where: Y = tissue or fat depot X = carcass weight)

Table 1.9 *Relative growth of the carcass tissue and fat depot*

Tissue (*Y*)	Growth coefficient (*b*)	SE
Lean	0.826	0.026
Bone	0.568	0.043
Subcutaneous fat	1.985	0.083
Intermuscular fat	1.006	0.039
Kidney knob and channel fat (KKCF)	1.680	0.102
Caul fat	1.868	0.196

(From Wood *et al.* 1980)

These values show that bone was the earliest maturing tissue, and subcutaneous fat was the latest while the intermuscular fat was earlier maturing than subcutaneous fat and both internal fat depots. In practical terms, this data clearly demonstrates that as the animal progresses towards maturity, its capacity for lean deposition becomes increasingly constrained by intrinsic factors. Wood *et al.* (1980) however pointed out that although type of breed did not affect composition in terms of the major tissues, it had an important effect on the percentage kidney knob and channel fat (KKCF) and caul fat. The ewe breeds had a higher KKCF and caul fat in relation to carcass weight and total fat. Studies with notably prolific sheep breed such as the Finnish Landrace, had earlier reported a large amount of internal fat relative to total fat (McClelland and Russell, 1972). Wood *et al.* (1980) concluded that internal fat deposition in sheep was related to the type of breed and suggested that prolific breeds probably require large deposits of internal fat as an energy reserve.

1.12.2 Breed differences

There are over, 50 breeds of sheep in the United Kingdom, all of which have characteristics which suit them for particular production systems. The breeds can broadly be classified into two types, for example the Hill or Down, Longwool or Shortwool breeds (Wood *et al.* 1980). Within the population used for meat production there are two distinct types which can be termed as the ewe breeds noted for prolificacy and milking ability, and the ram breeds noted for meat characteristics. Animals reared for slaughter are commonly crosses between these two types or between a crossbred female and male from one of the ram breeds. Ellis *et al.* (1997), compared three sire breeds, the Suffolk, Charolis and Texel. The authors reported that at the same estimated subcutaneous fat level, Suffolk sired lambs, were heavier at slaughter (mean live weight of 42.2, 41.0 and 40.7 kg for Suffolk, Charolis and Texel, respectively), despite having a similar killing out proportion compared to the other two breeds. Texel sired lambs had a higher lean proportion and lower total carcass fat compared to the other two breeds.

In their study evaluating ten sire breeds (Border Leicester, Dorset down, Hampshire Down, Ile de France, North Country Cheviot, Oxford Down, Southdown, Suffolk, Texel and Wensleydale), Kempster *et al.* (1987), observed that slower growing breeds, such as the Oxford Down, Border Leicester and Wensleydale were not suited to production systems requiring rapid growth, but instead fast growing breeds such as the Suffolk and Texel crosses were more suited to such production systems. The study demonstrated that there was considerable scope to increase carcass weights at a given level of fatness by breed substitution, although in some cases this would be at the expense of growth and increased time to slaughter. However, with the exception of the Texel (among the 10 sire breeds considered), there was less potential to increase carcass lean proportion at a given level of fatness (Kempster *et al.* 1987).

McClelland and Russell, (1972) observed that over the weight range of 26 to 41 kg, the total chemical fat deposited in relation to fleece free empty body weight was similar for both the Scottish Blackface and Finnish Landrace breeds. However, the Scottish Blackface was found to deposit relatively more fat muscular plus associated fatty tissues, than the Finnish Landrace, while the reverse was situation occurred in respect to of fat deposition in omental plus mesenteric and perirenal fatty tissues. Since, the two breeds have a similar mature body

weight of around 51 kg, the differences observed were due differences in patterns of fat deposition. The authors argued that when different sheep breeds were slaughtered at the same degree of maturity (proportion of mature size) their carcass composition would be similar. They clearly demonstrated this hypothesis using four breeds of sheep (Soay, Finnish Landrace, Southdown and Oxford Downs) whose mature size varied from, 25 to 100 kg live weight (McClelland *et al.* 1976). Comparisons were made at about 40, 50, 60 and 70 %, of their estimated mature body weight. Most of the breed differences in carcass composition observed at the same degree of maturity disappeared when expressed as a percentage of body or carcass weight. However, the percentage of fat differed between breeds, with the Soay having significantly less fat than the other breeds. Total muscle as a percentage of body weight was close to 28.5 % in all breeds at all four stages of maturity.

Hammond (1932), states that breeds such as the Downs breeds, which have been selected for meat production, have considerably more fat (principally in the subcutaneous depot) relative to body weight when mature than do wild or semi wild breeds such as the Soay and Shetland. These latter breeds have some similarities in conformation to both the Scottish Blackface and the Finnish Landrace, neither of which has been selected intensively for meat qualities. Such a hypothesis maybe supported by data presented by Butterfield *et al.* (1983), from a study where a large mature size strain of Merinos was slightly fatter at maturity than a smaller strain.

The retail value and lean content of the carcass may be improved in sheep at specified levels of total body fat when breeds which partition more of their body fat intra-abdominally at commercially acceptable weights are identified. McClelland and Russell, (1972) observed that the Finish Landrace tended towards more intra-abdominal fat, compared with the Scottish Blackface in a study where omental and mesenteric depots and intra- and intermuscular fat depots were combined in the measured chemical fat. Butler-Hogg (1984), observed a greater proportion of intra-abdominal and omental fat in the Clun lambs at each age (50, 100, 150 and 200 days of age) compared to lambs from the Southdown breeds which suggests a real breed difference related to breed type, as suggested by Wood *et al.* (1980).

1.12.3 Genetic selection for lean

Selection of terminal sire breeds is an effective method of improving lean meat production, because selection can be concentrated on a numerically small group of animals

which have a large genetic contribution to the market product (Cameron and Bracken 1992 and Bishop, 1993). But a substantial reduction in carcass fat content may have negative consequences on aspects of meat quality. For example selection for increased lean growth in pigs has been accompanied by a reduction in meat quality, largely due to a reduction in subcutaneous fat cover (Kempster *et al.* 1986). A reduction in carcass fat content in sheep is required for efficient lean meat production, but continued selection may result in substantial changes in fat quality. Additionally, selection for reduced carcass fat may have deleterious effects on the hill ewe productivity because hill breeds are required to deposit and mobilise quantities of body fat in response to nutritional constraints from the environment (Russell *et al.* 1968). Russell *et al.* (1968) demonstrated that the Hill ewe looses proportionally up to 0.20 of her live weight, including 0.85 of her subcutaneous fat reserves, during pregnancy and lactation. This propensity to fatness presents a problem not only to hill sheep farmers, who have to market undesirable products, but also to the United kingdom sheep industry because of the numerical importance of the hill breeds, and the effects they have on the sheep industry as a whole (Bishop 1993).

Studies on lines of Scottish Blackface, (Bishop, 1993) and Texel Oxford (Cameron and Bracken, 1992) sheep which had been divergently selected for predicted carcass lean content, have provided information on the effect of selection on fatty acid composition of the adipose triacylglycerides. Lipid content of the subcutaneous fat and lipoprotein activity were highly correlated, and both were positively correlated with the performance test traits in backfat thickness (Cameron *et al.* 1994). Additionally, the proportion of C18:2*n*-6 (linolenic acids) in the tissue triacylglycerides (TAG) was found to be positively correlated to backfat depth and the lipoprotein lipase activity of the adipose tissue. Speake *et al.* (1997) reported that the mean proportion of C18:2*n*-6 in the triacylglycerol of subcutaneous backfat was 1.3 fold higher in the phenotypically fatter sheep in the fat lines than in the phenotypically leaner sheep in the lean lines. Regression analysis indicated a positive relationship between this fatty acid and backfat depth whereas the proportion of C18:1*n*-9 (oleic acid) in the tissue TAG was negatively correlated. The authors suggested that plasma C18:2*n*-6 may have potential as a predictor (juvenile) of future fatness.

1.13 ANTIOXIDANTS IN MEAT QUALITY

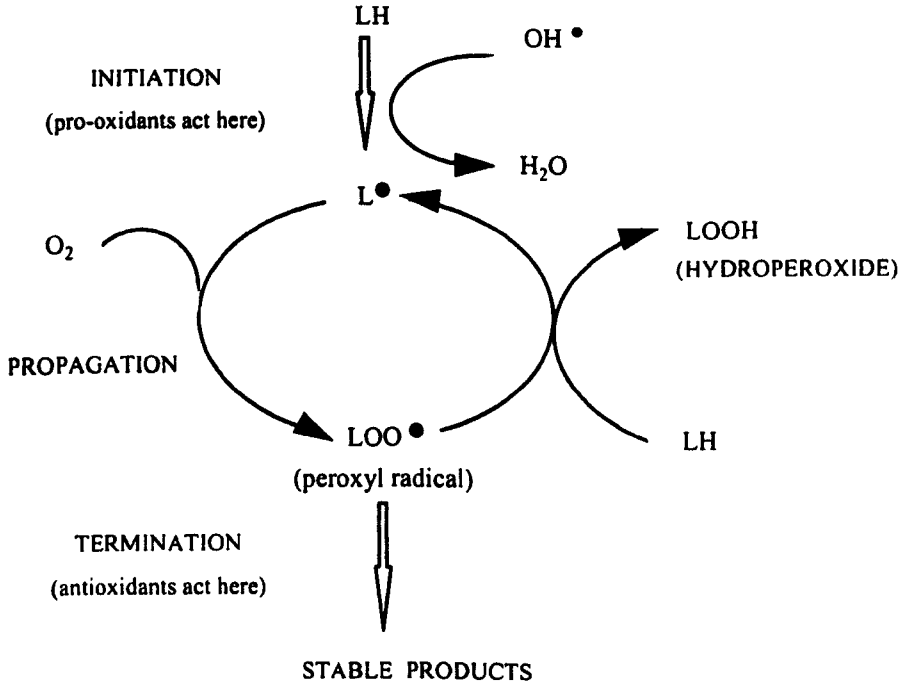
Dietary polyunsaturated fatty acids (PUFA) increase membrane unsaturation, as demonstrated in ruminants fed protected fats and consequently increases the susceptibility of meat to oxidation (Wood and Enser, 1997). Vitamin E is the major lipid soluble antioxidant in animal tissues which acts post-mortem to delay oxidative deterioration of meat, which manifests as a development of rancid odours and flavours from the degradation of PUFA in the membrane lipids (Hamilton, 1983).

Initiation : Production of free radicals

Propagation: $R + O_2 \Rightarrow ROO\bullet$

$ROO\bullet + RH \Rightarrow ROOH + R\bullet$

Termination: $ROO\bullet + ROO\bullet \Rightarrow$ molecular products



LH is the lipid chain; L• is a carbon-centred free radical; LOO• is a lipid peroxyl radical

Figure 1.25 The initiation, propagation and termination steps of chain reaction

(Adapted from Macpherson, 1994)

1.13.1 Lipid peroxidation mechanism

Lipid peroxidation occurs in three stages, as initiation, propagation and termination (Figure 1.25) (Macpherson, 1994). The first stage in oxidation is the loss of a hydrogen atom from the fatty acyl chain to give a free radical, which rapidly reacts with the oxygen to form a peroxy radical. The peroxy radical extracts a hydrogen from another hydrocarbon chain leaving an acyl radical and producing a hydrogen peroxide, which subsequently undergoes homolytic cleavage to give an acyloxy radical and a hydroxide radical. The breakdown of the acyloxyradical produces saturated and unsaturated aldehydes, alcohols, alkanes and alkenes. Further oxidation may occur on the original peroxides or on the unsaturated aldehydes, which then undergo further degradation. The reaction is now self-propagating and different peroxides maybe be produced which give rise to malonaldehyde when heated with acid in the thiobarbituric acid (TBA) method for determining rancidity (Vynke, 1976).

1.13.2 Vitamin E antioxidant mechanism

Vitamin E is the generic name for a group of lipid soluble compounds known as tocopherols and tocotrienols (tocols) (Rice and Kennedy, 1988). All isomers act as antioxidants to varying degree, the most powerful in this respect being α-tocopherol. The chemical structure of vitamin E has a 2-methyl-6-chromanol aromatic ring to which a 16 carbon saturated phytol chain is attached at C-2 (Figure1.26). The ring maybe methylated at C5, C7 and C8. The tocotrienols differ from the tocopherols in that the phytol chain contains 3 unsaturated double bonds. The degree of methylation of the chromanol ring results in four different isomers, i.e. alpha (α), beta (β), gamma (γ) and delta (δ) (Table 1.10). The greater the number of methyl groups on the chromanol ring the more efficient the molecule as an antioxidant.

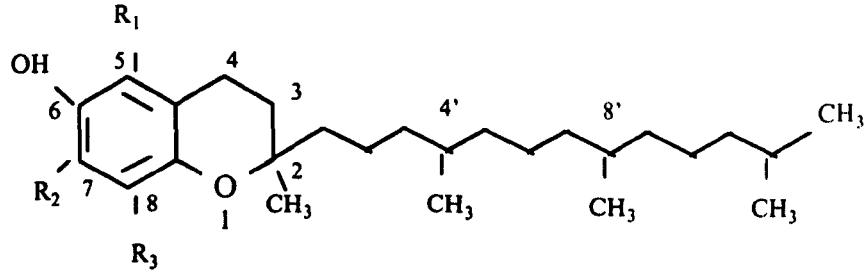


Figure 1.26 Structure of natural tocopherol.

The substituents of the different isomer (α, β, γ and δ) on the chromanol ring given in Table 1.10

Table 1.10 *Tocopherols*

	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Although all isomers appear to be absorbed (Peake and Beiri, 1971), they also appear to be rapidly excreted. Studies in livestock animals have shown that, although α-tocopherol, it was the only vitamin E isomer to occur in substantial quantities in blood and tissue (McMurray and Rice, 1982). The critical structure component of vitamin E as a free radical scavenger is the hydroxyl group, which is common to all tocopherols and tocotrienols. The methyl groups on the chromanol ring and the structure of the side chain (phytol chain) are important in the localisation of the hydroxyl group in the subcellular membranes (Rice and Kennedy, 1988).

1.13.3 Vitamin E requirement

Recommended allowances by both the Agricultural Research Council (1980) and the National Research council (1989) in all ruminants are: 15 to 40 mg/kg DM vitamin E. When dietary polyunsaturated fats are offered to pre-ruminant calves and lambs, additional vitamin E should be given at 3 mg/g of PUFA in the diet to prevent muscular dystrophy. Recommendations have been given for both ruminant and non-ruminant animals (Putman and Comben, 1987), based on a mathematical relationship between the input of dietary PUFA and vitamin E requirement. There is however, controversy regarding these recommendations and recent research suggests that relatively high levels (40-70 mg/kg DM) of supplemental vitamin E in ruminants may improve performance in terms of immunity (Reddy *et al.* 1987; Nockels, 1991 and Njeru *et al.* 1994) and supra nutritional levels (500 mg/kg DM) may improve carcass quality (Schaefer *et al.* 1991).

Dietary Vitamin E supplementation results in elevated concentrations of α-tocopherol in the cell membrane, especially in the mitochondria and the microsomes, resulting in a significantly lower susceptibility to lipid oxidation in the membrane (Ashgar *et al.* 1991). Monahan *et al.* (1990), reported that dietary supplementation (up to 200 mg/kg feed α-tocopherol acetate)

significantly improved the oxidative stability of both raw and cooked pork muscle during storage at 4°C for up to 8 days, and in addition stabilised the membrane bound lipids against oxidation. The authors concluded that the incorporation of vitamin E into membrane lipid via the diet, was the most effective means of extending shelf life in pre-cooked meats products. Cannon *et al.* (1995) also reported that supplementation of vitamin E (100 mg/kg feed) in the swine diet provided added protection against lipid peroxidation, and pre-cooked pork under vacuum provided a palatable product with a shelf life greater than 56 days. Faustman *et al.* (1989) reported improved lipid stability in beef from Holstein steers whose diets were supplemented with 370 IU vitamin E per animal per day for approximately 43 weeks. Arnold et al (1993b), obtained similar results after supplementing Holstein steers with 0, 360 and 1290 IU daily for 6 months. In the latter study, lipid oxidation was markedly inhibited by the 360 IU supplement, leaving no margin for further improvement by the highest level of supplementation. The authors observed that α-tocopherol concentrations in excess of 3 mg/kg meat (fresh basis) were necessary for reducing lipid oxidation in ground beef. Meat cuts containing α-tocopherol in excess of this concentration do not appear to have any benefit in terms of reduced lipid oxidation (Faustman *et al.* 1989a), although liver and subcutaneous fat seem to have the capacity to accumulate more α-tocopherol at higher level of supplementation (Arnold *et al.* 1993a).

Studies reporting the effects of vitamin E supplementation on lipid oxidation and meat quality in sheep are limited. Wulf *et al.* (1995) reported that feeding sheep 500 mg α-tocopherol per day improved lipid stability and colour shelf life for up to four days, when the tissues levels of vitamin E were in excess of 5.5 mg/kg. Of more importance is the effectiveness of vitamin E, in conjunction with selenium to counteract white muscle disease (WMD) or nutritional muscular dystrophy (NMD) in both young lambs and calves (McMurray *et al.* 1980 and McDowell *et al.* 1985). Rice *et al.* (1981), showed that linolenic acid (C18:3*n*-3), if protected from ruminal hydrogenation, rapidly reached high levels in blood, and was associated with a rise in plasma creatine kinase, indicating muscular degenerative myopathy.

1.13.4 Selenium and vitamin E interactions

Selenium prevents lipid oxidation due to its presence in the enzymes glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase (Macpherson, 1994). Animals deficient in both selenium and vitamin E suffer from nutritional degenerative

myopathy, nutritional muscular deficiency or white muscle disease (Rice and Kennedy, 1988), whilst deficiency of selenium alone with adequate vitamin E, does not result in increased lipid oxidation. Excessive selenium is toxic and the recommended dietary levels are given as 0.1-0.2 mg/kg DM for ruminant (ARC, 1980).

1.14 CONCLUSION

Ruminant meats and meat products are high in saturated fatty acid, which have been associated with increased risk to cardiovascular diseases, whilst longer chain *n*-3 PUFA, especially C20:5 and C22:6 are considered to be effective in reducing the morbidity of coronary heart diseases. Manipulating the fatty acid composition of ruminants to meet modern nutritional guidelines has its limitation due to rumen biohydrogenation. The aims of the current study were to quantify the duodenal flow of dietary *n*-3 PUFA from different sources and investigate their effects on muscle and adipose tissue fatty acid composition in different sheep breeds. The effects of dietary vitamin E level on tissue vitamin E were also investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 ROUTINE ANALYSIS

2.1.1 Dry matter (DM)

Sub-samples of pelleted rations were bulked and ground through a 1 mm mill (Retsch zm- 1000) before being dried to constant weight at 80°C. Faecal samples were thawed and bulked before blending with a food processor (Tecator Homogenizer-1094) and drying (10 % of the fresh weight) to constant weight at 80°C. Duodenal digesta, isolated rumen microbes and pooled faecal samples were frozen and freeze dried (Edwards 4K Modulyo freeze dryer) to constant weight before storing in airtight containers at minus 20 °C.

2.1.2 Organic matter (OM)

Organic matter content of the feed, digesta and faecal samples was determined by weighing 2 g of dried sample into a preweighed silica crucible and ashing at 550°C overnight in a muffle furnace (Gallenkamp Muffle furnace Size 3 GAFSE 620). Ashed samples were cooled in a desiccator to room temperature and the weight of residue recorded as the ash content. Organic matter was calculated as described below (AOAC, 1980).

$$OM\ (g/kg\ DM) = 1000 - ash$$

2.1.3 Nitrogen analysis

2.1.3.1 Total nitrogen

Total Nitrogen of the feed, digesta and faecal samples was determined using the Kjeldahl digestion procedure. Approximately 0.5 g of feed, duodenal digesta and faeces or 0.05 g of isolated rumen microbes samples were weighed in duplicate into nitrogen free filter paper envelopes and digested at 450°C for 60 minutes using 14 ml of concentrated sulphuric acid and copper sulphate as a catalyst. After cooling to room temperature the digest was automatically made alkaline with sodium hydroxide and distilled into a solution of 4 % boric

acid before titrating with 0.1 M hydrochloric acid (Kjeltec auto sampler 1035 analyser-Tecator). The nitrogen content of the sample was calculated using the sample weight and the volume of acid required to neutralise the ammonia released as described below:

$$1 \text{ ml of } 0.1 \text{ HCl} = 0.0014 \text{ g Nitrogen}$$

$$\text{Nitrogen } (\text{g/kg}) = \left(\frac{1.4 \text{ x volume of HCl}}{\text{Sample Wt (g)}} \right)$$

A factor of 6.25 was used to convert the feed nitrogen to crude protein.

2.1.3.2 Duodenal digesta ammonia nitrogen

Ammonia nitrogen of the duodenal digesta samples was determined by weighing 0.5 g of freeze dried sample into 15 ml centrifuge tubes in duplicates. To each tube was added 5 ml of 0.2 M hydrochloric acid and the samples were left to stand for 30 minutes at 4°C. The samples were then centrifuged at 1000 g for 10 minutes and the supernatant filtered (Whatman No. 1 filter paper). The filtrate was then diluted 1:10 using 0.02 M hydrochloric acid and ammonia nitrogen determined by distilling 10 ml of the sample solution with 6 ml magnesium oxide suspension and titrating with 0.02 M hydrochloric acid using a manual analyser (KjeltecTecator 1030). Quantification was based on an external standard prepared using oven dried ammonium sulphate and 0.02 M hydrochloric acid with a calibration curve ranging from 5 to 25 ppm ammonia nitrogen.

2.1.4 Neutral detergent fibre (NDF)

Neutral detergent fibre of feed, digesta and faecal samples was determined by the methods of Goering and Van Soest (1970). Into a preweighed crucible 0.5 g of dried sample was weighed and digested for 30 minutes with 25 ml neutral detergent solution [93 g disodium ethylene tetra-acetate dihydrate (EDTA), 34 g sodium borate, 150 g sodium lauryl sulphate, 50 ml 2-ethoxy ethanol mixed with 22.8 g anhydrous disodium phosphate made up to 5 litres and pH adjusted to 7] and 0.5 ml octanol using the Fibertech apparatus (Fibertec System M-Tecator). After digestion, 2 ml -amylase solution (2 g α-amylase E.C.3.2.1.1 from *Bacillus subtilis* in 90 ml water filtered and 10 ml 2-ethoxy ethanol added stored at 4°C) was added and the digestion procedure repeated before filtering and washing the digest with 3 x 20 ml hot water. To the digest was then added 25 ml hot water (80°C) and 2 ml -amylase and the sample left to stand for 15 minutes before repeating the washing procedure and drying with 20

ml acetone. The crucible with digested sample was oven dried at 100°C overnight and cooled in a desiccator before reweighing and ashing at 550°C for 4 hours and the ash weighed. The neutral detergent fibre content was calculated as described below;

$$\text{NDF weight (g)} = [(\text{crucible (g)} + \text{dry fibre weight (g)}) - (\text{crucible (g)} + \text{ash weight (g)})]$$

$$\text{NDF (g/kg DM)} = \left(\frac{\text{NDF weight (g)}}{\text{Sample weight (g)}} \right) \times 1000$$

2.2 MEASUREMENT OF DIGESTA FLOW TO THE DUODENUM

Estimates of rate of digesta flow at the duodenum were obtained using a dual phase marker technique with ytterbium acetate as the particulate marker and chromium-EDTA as the liquid phase marker (Faichney, 1975).

2.2.1 Preparation and administration of digesta flow markers

Chromium-EDTA was prepared using the method of Binnerts *et al.* (1968). During preparation 56.8 g of pure chromium-trichloride ($CrCl_3.6H_2O$) and 80 g disodium salt of ethylenediaminotetracetic acid (Na_2-EDTA) were boiled for one hour in 1500 ml distilled water until a deep violet colour was produced and a 1:1 complex of EDTA:Chromium formed. Excess EDTA was neutralised with 16 ml of 1M calcium chloride and the solution adjusted to pH 7 using concentrated sodium hydroxide. This was then filtered and made up to 2000 ml using distilled water. This provided a concentration of 5.43 mg chromium per ml, which was diluted with distilled water to 1.3125 mg per ml prior to infusion to provide an infusion of 140 mg chromium/ kg dry matter intake. Ytterbium acetate (Sigma-Aldrich Co. Ltd Dorset) was dissolved in distilled water to provide an infusion of 50 mg ytterbium/ kg dry matter intake.

Markers were infused via separate lines into the reticulo-rumen using a peristaltic pump (Watson Marlow-205U) at a rate of 4.5 ml per hour. The infusion tubing, 0.86 mm internal diameter (Phillip Harris Scientific) was connected to a 3 mm internal diameter rubber tubing at point of entry into the rumen cannulae. A branched 'Y'-tubing measuring approximately 30 cm was used to disperse markers in the rumen. An initial priming dose of 40 ml, of both markers was given to bring marker concentration in the rumen close to plateau.

2.2.2 Rumen sampling

Rumen fluid samples of approximately 80 ml was taken via the rumen cannula using a manual vacuum pump and filtered through two layers of muslin into 50 ml centrifuge tubes. The initial 40 ml rumen fluid was considered unrepresentative of the rumen fluid and discarded.

2.2.2.1 Isolation of rumen microbes

Rumen microbial isolates were separated from strained rumen fluid by a modified method from Mathers and Miller (1980). Rumen fluid samples were centrifuged (Beckmans

Avanti- 30 HP) at 1 000 g for 5 minutes at 4°C to separate feed particles. The supernatant was then decanted into a clean centrifuge tube and recentrifuged at 28 600 g for 20 minutes at 4°C to isolate microbial cells. The supernatant was then decanted and the pH recorded before acidifying (3 drops concentrated HCL) the samples and storing at minus 20°C for subsequent volatile fatty acid (VFA) analysis. The isolated microbial cells were re-suspended in McDougall's buffer (9.8 g/l $NaHCO_3$, 3.7 g/l Na_2HPO_4, 0.47 g/l NaCl, 0.57 g/l KCL, 0.04 g/l anhydrous $CaCl_2$ and 0.06 g/l anhydrous $MgCl_2$) and re-centrifuged at 28 600 g for 20 minutes. The residue was then frozen and freeze-dried prior to subsequent analysis.

2.2.3 Duodenal sampling

In order to achieve equilibrium, digesta flow markers were infused for 8 days prior to the collection of duodenal digesta samples. Approximately 100 ml of duodenal digesta was collected by gravity from the 'T'- piece cannula. The initial 10 ml digest was considered unrepresentative of flow and discarded. Four digesta samples were taken daily from each animal over a 3 days period, pooled together and stored at minus 18°C. After the collection period bulked samples were thawed and thoroughly mixed before taking half of the bulked digesta sample and freeze drying to represent whole duodenal digesta (WDD). The remaining fraction was centrifuged at 1000 g for 5 minutes and the supernatant discarded to obtain a particulate duodenal digesta sample (PDD) which was frozen and freeze-dried.

2.2.4 Analysis of digesta flow markers

Chromium and ytterbium were analysed in both the whole and particulate digesta by the methods of Siddons *et al.* (1985).

2.2.4.1 Chromium

Chromium was determined by weighing 0.5 g of freeze-dried digesta into a 50 ml conical flask (Pyrex) and ashing at 450°C for 16 hours in a muffle furnace. After cooling 6 ml digestion acid (250 ml orthophosphoric acid (880 g/l), 50 ml manganese sulphate (100 g/l), and 250 ml concentrated sulphuric acid made up to 1 000 ml with distilled water) was added to the ash and the mixture boiled on a hot plate. On boiling, 3 ml potassium bromate (45 g/l) was added and the boiling continued until the solution turned a deep purple colour. The solution was then made up to 100 ml with distilled water and the chromium content was measured using atomic absorption spectrophotometry (Smith-Hieftje 1000). Quantification was based

on an external standard made up using potassium dichromate. The calibration curve ranged from 5 to 15 mg/l chromium. Chromium content of the infusion solution was determined by direct aspiration after appropriate dilution. The full operating conditions of the atomic absorption spectrophotometer are presented in Table 1.

2.2.4.2 Ytterbium

Ytterbium was determined by weighing 0.5 g of the freeze-dried digesta sample into a 50 ml Pyrex conical flask and ashing at 450°C for 16 hours. Ytterbium was extracted from the ashed samples by adding 10 ml of 2 % v/v nitric acid containing 1 mg/ ml potassium chloride and mixing for 2 hours on a rotary shaker. The sample solutions were then transferred into 25 ml centrifuge tubes and centrifuged at 1100 g for 10 minutes. Ytterbium was determined in the supernatant using atomic absorption spectrophotometry. Quantification was based on external standards prepared in blank digesta containing similar matrix interference as the samples by adding 15, 30 and 50 µg ytterbium in 5 ml of distilled water to 0.5 g blank digesta. Standards were oven dried overnight and the ytterbium extracted as described previously to provide working standards of 1.5, 3 and 5 µg/ ml ytterbium.

Ytterbium acetate infusion solutions were diluted in digestion acid to obtain a final concentration of 2 % nitric acid and 1 mg/ ml potassium. These were analysed against a set of standards covering the range of 0-5 µg/ ml prepared in the same concentration of acid. The full operating conditions of the atomic absorption spectrophotometer are presented in Table 1.

Table 2.1 *Atomic Absorption operating conditions*

	Chromium	Ytterbium
Wavelength (nm)	357.9	398.8
Spectral band pass (nm)	0.4	0.4
Fuel	Acetylene	Acetylene
Support	Nitrous oxide	Nitrous oxide
Lamp (mA)	6	5

2.2 5 Dry matter flow calculation

Dry matter flow to the duodenum was calculated from the liquid and the solid phase marker concentrations in the whole duodenal digesta (WDD) and particulate duodenal digesta (PDD) using the equation of Faichney (1975a). Ytterbium acetate (Yb) was assumed to be

100 % recoverable at the duodenum and Chromium (Cr) 95 % recoverable (Siddons *et al.* 1985).

Yb and Cr concentrations in both the WDD and PDD were expressed as a proportion of the daily dose of the marker;

$$R = \left\langle \frac{[\text{Yb of WDD}] - [\text{Cr of WDD}]}{[\text{Cr of PDD}] - [\text{Yb of PDD}]} \right\rangle$$

Where R = factor required to mathematically reconstitute the complete duodenal digesta (CDD).

Then:

$$[\text{Cr of CDD}] = [\text{Cr of WDD}] + \left[R \times \frac{[\text{Cr of PDD}]}{1 + R} \right]$$

Then:

$$\text{DM flow (g / day)} = \left[\frac{1}{\text{Cr in CDD}} \right]$$

2.2.5.1 Nutrient flow calculation

Samples of WDD and PDD were analysed for, organic matter, nitrogen, neutral detergent fibre, microbial marker (purine) fatty acids and ammonia nitrogen. The concentration (g/kg DM) was calculated using the equation of Faichney (1975a):

$$[\text{X of CDD}] = \left[\frac{[\text{X of WDD} + (R \times [\text{X of PDD}])]}{1 + R} \right]$$

Where: X = Nutrient composition

$$\text{Flow (g/day) of each nutrient} = [\text{X of CDD}] \times \text{DM flow (g/day)}$$

2.3 RUMEN VOLATILE FATTY ACIDS ANALYSIS

Volatile fatty acids were analysed according to the method of Ryan (1980). Frozen acidified rumen fluid samples were thawed at room temperature and thoroughly mixed before transferring 10 ml into 50 ml centrifuge tubes and centrifuging at 28 600 *g* for 30 minutes at 4°C. A sample of 4.5 ml of the supernatant was then transferred into 15 ml soveril tube and 0.5 ml of internal standard (25 mM phenol) added. The sample was vortexed for 10 seconds and then filtered through a 0.2 μm pore size syringe filter (Whatmans nitrocellulose membrane filter) into vials ready for gas chromatography.

Volatile fatty acids were analysed by gas liquid chromatography on a 30m x 0.25mm internal diameter (DB-FFAP) column. The chromatograph was a Perkin-Elmer 8500 with an AS3800 autosampler with flame ionisation detection and helium as the carrier gas (split 5:1). A 2μl samples was injected at 300°C. The column temperature was 110°C and increased at 10°C per minute up to 200°C where it was held for 17 minutes.

Volatile fatty acids were quantified using an integrator and phenol as an internal standard. Each major peak was identified by comparison of retention times with those of known standards. Linearity of response was confirmed using external standards of known concentrations of volatile fatty acids

2.4 DETERMINATION OF TOTAL PURINES

The total purine content of isolated bacterial cells and duodenal digesta was determined according to the methods of Zinn and Owen (1986).

2.4.1 Perchloric acid hydrolysis

Freeze dried samples of 0.06 g isolated microbial cells and 0.5 g duodenal digesta were weighed accurately in duplicate into 25 ml screw cap culture tubes and 2.5 ml of 70 % perchloric acid added before incubating samples at 95°C in a water bath for 60 minutes. At the same time 20, 40 and 60 mg of yeast ribonucleic acid (RNA) standard (Torula Yeast Type VI, R6625, Sigma Chemical Co.) was carried through the procedure. The pellet was broken and 17.5 ml of 0.0285M ammonium di-hydrogen orthophosphate ($NH_4H_2PO_4$) buffer was added and the mixture vortexed and re-incubated at 95°C for a further 15 minutes. After cooling the sample was filtered through glass fibre filter paper (Whatman GF/A) and 0.5 ml of the filtrate transferred into a 15 ml centrifuge tube and 0.5 ml of 0.4M silver nitrate and 9 ml buffer (0.2M $NH_4H_2PO_4$) was added before leaving the sample to stand in the dark overnight at 5°C. The sample was then centrifuged at 1000 g for 15 minutes and the supernatant decanted with care so as not to disturb the pellet. The pellet was washed with 0.2 M $NH_4H_2PO_4$ and vortexed before centrifuging and discarding the supernatant. To the pellet was added 10 ml of 0.5M hydrochloric acid and the sample vortexed before transferring into a clean 15 ml tube.

The samples were then covered with a marble and incubated at 95°C for 30 minutes in a water bath. After allowing cooling the sample was the filtered through a coarse filter paper (Whatman No. 4).

2.4.2 Analysis of total purines

Absorbency of the supernatant was read at 260 nm against a blank solution on a spectrophotometer (Beckman DU 600). The final concentration of RNA equivalents (purine bases) in the standards were 0.5, 1.0, 1.5 mg/ ml relating to 20, 40 and 60 mg RNA originally used. A standard regression relating absorbency (X) to concentration (Y mg/ ml) was calculated. The recovery of total purine was determined by adding known amounts of RNA and calculating as presented in Table 2.2.

Table 2.2 *Recovery of purine (mg Purine N/g DM)*

Yeast RNA added	Total Purine content	% Recovery
-	3.45	-
1.02	4.59	103.0
1.49	4.90	98.9
2.38	5.83	100.0
	Mean	100 ± 0.6

2.4.2.1 Calculating microbial efficiency

Non ammonia nitrogen (NAN) at the duodenum was calculated as follows

Duodenal NAN flow (g/day) = [Duodenal N flow (g/d) - duodenal ammonia - N flow (g/d)]

The RNA equivalence (Purine bases) was used to calculate microbial nitrogen as follows;

$$\text{Microbial N flow (g/day)} = \left[\frac{\left(\frac{\text{Concentration of purine bases in duodenal digesta (mg/g DM)}}{\text{Concentration of NAN in duodenal digesta (mg/g DM)}}\right)}{\left(\frac{\text{Concentration of purine bases in microbial isolate (mg/g DM}}{\text{Microbial isolate N concentration (mg/g DM)}}\right)}\right] \times \text{NAN flow (g/day)}$$

Apparent microbial efficiency was calculated as microbial nitrogen per kg organic matter apparently digested in the rumen as follows;

$$\text{Apparent Microbial efficiency (g N/ kg OMADR)} = \left[\frac{\text{Duodenal microbial nitrogen flow (g/d)}}{\text{OM intake (g/d) - Duodenal OM flow (g/d)}}\right]$$

True microbial efficiency was calculated as microbial nitrogen per kg organic matter truly digested in the rumen (OMTDR) as follows;

$$\text{True Microbial Efficiency (g N/kg OMTDR)} = \left[\frac{\text{Duodenal microbial N flow (g/d)}}{\text{(OM intake (g/d) - Duodenal OM flow (g/d))} + \text{Duodenal microbial OM flow (g/d)}}\right]$$

2.5 ANIMAL SLAUGHTERING AND TISSUE SAMPLING PROCEDURE

All animals were slaughtered at the University of Bristol abattoir. Sheep were fasted overnight prior to slaughter to minimise contamination during slaughter.

2.5.1 Slaughter and dressing procedure

Electric stunning and exanguination were used to effect slaughter. Feet were then removed, leaving the carpals and tarsals on the carcass. After skinning, the head was separated from the carcass between the occipital bone and the first cervical vertebra. Evisceration of the carcass was done in the normal commercial practice and all major organs inspected. The carcasses were then hanged by the hind legs from a standard gambriel and the weight recorded as the "hot" carcass weight. The hanging carcass was removed into cold storage chambers for cooling at 1°C for 24 hours. Carcass pH was also recorded over the 24 hours period. After cooling the carcass weight was recorded as the "cold" carcass weight.

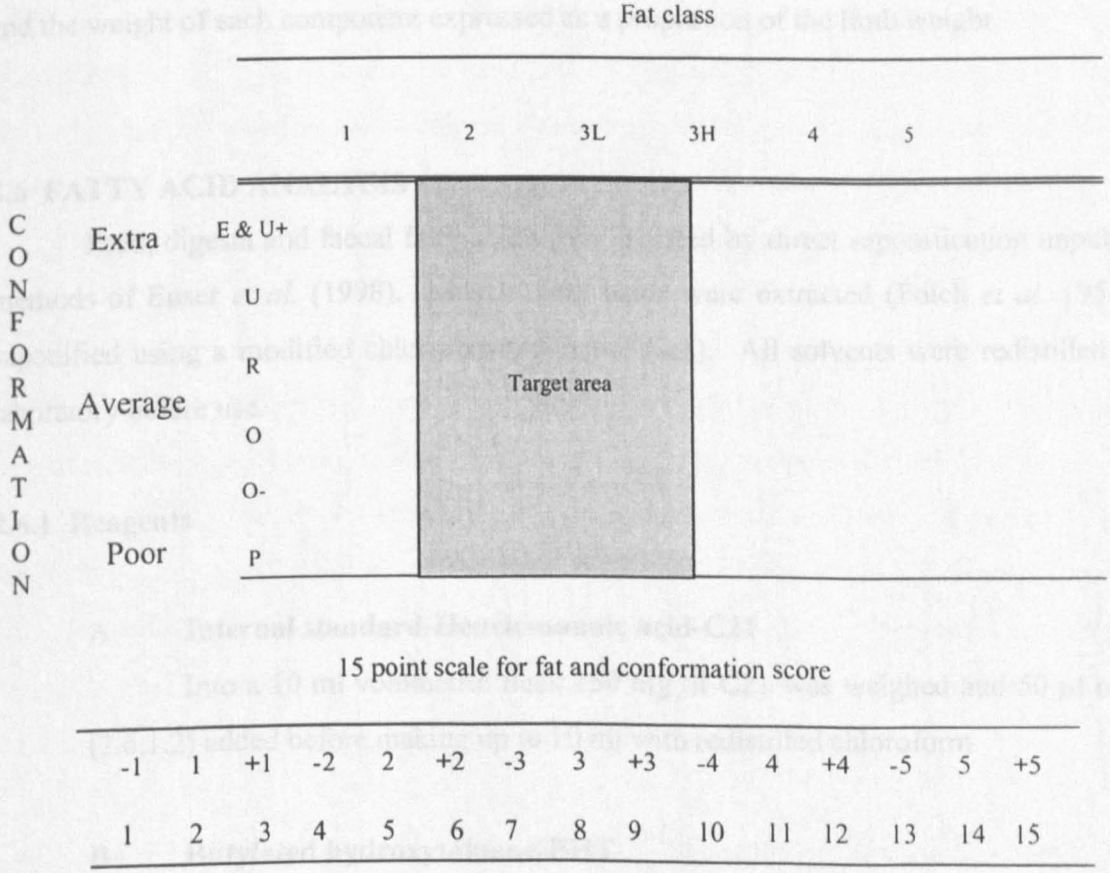


Figure 2.1 MLC and EU classification scheme

(Adapted from Kempster *et al.* 1986)

2.5.2 Carcass classification

External fat cover was estimated visually to the nearest percentage unit of subcutaneous fat in the carcass (SF_e) and conformation assessed using the Meat and Livestock Commission (MLC) classification scheme (1983) (Figure 2.1). The values were then converted to a 15-point scale using the European Union (EU) system (Kempster *et al.* 1986).

2.5.3 Sample collection

Adipose tissue samples of 5cm by 5cm were dissected from the loin of the cold carcass. The whole carcass was then split longitudinally into two equal halves. From the half carcass (left side), samples of *semimembranosus* and *longissimus dorsi* muscles were dissected from the hind limb and trimmed off all visible fat. Samples were then vacuum packed, frozen rapidly and stored at minus 20°C for subsequent analysis of fatty acids and α-tocopherol.

The forelimb was dissected (left side of the carcass), vacuum packed and frozen at minus 20°C. This was later thawed and dissected into lean, bone, subcutaneous and intermuscular fat and the weight of each component expressed as a proportion of the limb weight.

2.6 FATTY ACID ANALYSIS

Feed, digesta and faecal fatty acids were isolated by direct saponification unpublished methods of Enser *et al.* (1998). Muscle fatty acids were extracted (Folch *et al.* 1957) and saponified using a modified chloroform/methanol (2:1). All solvents were redistilled in the laboratory before use.

2.6.1 Reagents

A Internal standard-Heneicosanoic acid-C21

Into a 10 ml volumetric flask 150 mg of C21 was weighed and 50 µl of BHT (2.6.1.2) added before making up to 10 ml with redistilled chloroform.

B Butylated hydroxytoluone-BHT

Into a 100 ml volumetric flask 1 g BHT was weighed and made up to 100 ml with chloroform.

C Saponification mixture - 5 Molar potassium hydroxide (KOH)

Into a 250 ml measuring cylinder 140.3 g KOH was weighed and rapidly dissolved in 200 ml distilled water before capping the cylinder and leaving the solution to cool down to room temperature (the cylinder was held in a water bath under running cool water). After cooling the solution was made up to mark with distilled water. In a beaker 0.5 g of quinol was weighed and dissolved in a small amount of methanol before transferring into a 250 ml volumetric flask and making up with methanol. The two solutions were then mixed together (250 ml KOH plus 250 ml methanol with quinol).

D Saponification mixture - 2 M KOH

Into a 250 ml measuring cylinder 56 g KOH was weighed and rapidly dissolved in 200 ml distilled water before capping the cylinder and leaving the solution to cool down to room temperature (the cylinder was held in a water bath under running cool water). After cooling the solution was made up to mark with distilled water. In a beaker 0.5 g of quinol was weighed and dissolved in a small amount of methanol before transferring into a 250 ml volumetric flask and making up with methanol. The two solutions were then mixed together (250 ml KOH plus 250 ml methanol with quinol).

2.6.2 Feed fatty acid saponification and extraction

Feed samples were ground and mixed thoroughly before accurately weighing 0.4 g in duplicate into 30 ml soveril tubes. This sample weight was estimated to give approximately 20-25 mg of fat, which was required for an accurate chromatogram. To each sample was added 700 µl of distilled water and 100 µl of internal standard (2.6.1A), 50µl of antioxidant (2.6.1B) and 6 ml of a 5M saponification mixture (2.6.1C) and the samples saponified for 3 hours in a water bath held at 60°C with regular manual shaking. On cooling 3 ml of 10N sulphuric acid was added and samples saponified for a further 1 hour.

To the tubes were added 12 ml distilled water and 5 ml petroleum spirit (b.p.40-60°C) and the tubes manually shaken vigorously before centrifuging for 1-2 minutes at 1000 *g*. Absolute ethanol was added dropwise to clear the gel in the top layer before transferring the supernatant

using a Pasteur pipette into a clean soveril tube and the procedure repeated twice. Sodium hydrogen carbonate was then added until fizzing stopped and then anhydrous sodium sulphate until the powder fell through before centrifuging the sample for 5 minutes at 1000 *g*. The top layer was decanted into clean soveril tubes and stored at minus 18°C under oxygen free nitrogen ready for methylation.

2.6.3 Digesta and faecal fatty acid saponification and extraction

Freeze dried digesta and faecal samples were thoroughly mixed using a pestle and mortar before accurately weighing in duplicate 0.2-0.25 and 0.6-0.7 g of digesta and faecal samples, respectively, into 30 ml soveril tubes. To each sample tube was added 700 µl of distilled water, 100 µl of internal standard (2.6.1A) and 6 ml of 5M saponification mixture (2.6.1C). Samples were saponified for 2 hours and 30 minutes at 60°C, whilst shaking regularly. To each sample was then added 12 ml of distilled water and 5 ml petroleum spirit and the samples shaken vigorously before centrifuging at 1000 *g* for 3 minutes and adding absolute ethanol dropwise to clear the gel which was discarded and the procedure repeated twice.

To each sample tube 3 ml of 10N sulphuric acid and 5 ml petroleum spirit was added and the samples vigorously shaken manually, before centrifuging for 1-2 minutes at 1000 *g* and transferring the top layer using a Pasteur pipette to a clean soveril tube and repeating the procedure twice. Sodium hydrogen carbonate was added until fizzing stopped and then anhydrous sodium sulphate was added until the powder fell through before centrifuging the sample for 5 minutes at 1000 *g*. The top layer was decanted into clean soveril tubes and stored at minus 18°C under oxygen free nitrogen ready for methylation.

2.6.4 Muscle fatty acid extraction

Frozen muscle samples were partially thawed and trimmed off all visible fat and surface connective tissue before cutting into approximately $1cm^3$ pieces and blending in a mini food processor in short bursts and mixing where necessary to obtain a smooth paste.

Into a 250 ml conical flask a 9.95-10.05 g sample was accurately weighed and 200 µl of a 10 % solution of BHT in chloroform added as an antioxidant before adding 66 ml of methanol. The flask was capped and shaken to disperse the tissue sample before adding 132 ml

chloroform and blending using a Polytron mixer (PT-MR-3000 Kinematic) at approximately 20 000 rpm. The sample flask was then capped and shaken vigorously before leaving it to stand overnight in a dark cupboard to precipitate the protein.

The sample was filtered under suction through a filter paper (Whatmans 7 cm GFA) using a Hartely 3 piece funnel with Teflon support disk into a 250 ml Buchner (side arm) conical flask. The filtrate was transferred into a 250 ml screw capped measuring cylinder and the volume made up to 220 ml using a chloroform:methanol (2:1) mixture (volume noted if <220 ml). To the sample was added 44 ml of 0.58 % NaCl (w/v) (or 1/5 volume of the extract if <220 ml), after which the cylinder was capped inverted once to release the pressure and stored overnight in the dark to allow phases to separate.

The upper phase of the sample was aspirated and discarded taking care not to suck off the interfacial material after which 30 ml of absolute ethanol was added and mixed by inverting the cylinder. The sample was then transferred into a 250 ml round bottomed quickfit flask and dried at 60° C on a vacuum rotary evaporator (BUCHII-Rotorvapor). Upon drying approximately 30 ml of absolute ethanol was added immediately and the flask swirled and the drying procedure repeated. To the sample flask was added 10 ml of chloroform and the sample swirled before capping the flask and leaving to stand on the bench for 3-4 hours. The samples were then filtered under suction through a filter paper (Whatmans 4.7cm GFA) on a small 3 piece Hartley funnel into 30 ml soveril tubes and stored at minus 18°C.

Samples were brought to room temperature, anhydrous sodium sulphate added until the powder fell through before leaving the sample to stand for 30 minutes and filtering using a small vacuum filter through a filter paper (Whatmans 4.7 cm GFA) on a disc. The sample volume was reduced to approximately 8 ml in a 60°C water bath under oxygen free nitrogen before transferring the solution to a 10 ml volumetric flask and making up to the mark with chloroform. Samples were then transferred and stored in 10 ml Soveril tubes (the miniscus was marked incase of evaporation) at minus 18°C under oxygen free nitrogen ready for saponification.

2.6.4.1 Gravimetric fat weight of muscle sample

Weight of fat was determined (exact weight of fat per ml of solution) by transferring 2 ml of the extracted fat solution using a glass pipette onto a pre-weighed foil dish and leaving to stand in a fume cupboard before drying in the oven for 15 minutes and weighing.

$$\text{Fat weight (mg/ml)} = \left[\frac{(\text{Weight of dried sample + foil dish (g)}) - \text{Wt empty foil dish (g)})}{2}\right]$$

The amount of fat solution to be directly saponified was adjusted accordingly (20-25mg of fat was required for an accurate chromatogram).

2.6.4.2 Muscle fat saponification

Frozen extracted fat samples were brought to room temperature, mixed thoroughly and a volume equivalent to 20-25 mg fat transferred into a 15 ml soveril tube in duplicate. To each sample was added 100 µl of C21 (2.6.1a) before drying at 60°C under oxygen free nitrogen. Upon drying 2 ml of a 2M saponification mixture (2.6.1d) was immediately added and samples saponified for 1 hour in a 60°C water bath with regular vigorous shaking manually in the first half hour.

To the sample was added 7 ml of distilled water and 3 ml of petroleum spirit and the sample vigorously shaken manually before centrifuging for 1-2 minutes at 1000 g. Absolute ethanol was added dropwise to clear the gel in the top layer, which was discarded, and the procedure repeated twice. To the sample was then added 0.5 ml of 10M sulphuric acid and 3 ml of petroleum spirit and the sample vigorously shaken manually before centrifuging for 1-2 minutes at 1000 *g* and transferring the top layer using a Pasteur pipette into a clean soveril tube and repeating the procedure twice. The washed solution was tested for acidity (pH<2 required for complete extraction) and sodium hydrogen carbonate added until fizzing stopped and then anhydrous sodium sulphate until the powder fell through before centrifuging the sample for 5 minutes at 1000 *g*. The top layer was decanted into clean soveril tubes and stored at minus 18°C under oxygen free nitrogen ready for methylation.

2.6.5. Adipose tissue fatty acids

All layers of the subcutaneous fat were sampled and trimmed off any visible fleece muscle and connective tissue. Using a scalpel blade the tissue was roughly chopped and 1g

sample weighed in duplicate into a 30 ml soveril tube. To each sample was added 17 ml chloroform plus 100 µl of BHT (2.6.1b) and the sample blended using a Polytron mixer at approximately 20 000 rpm for 1 minute. The sample tubes were capped after adding 3 g sodium sulphate (anhydrous), and then shaken vigorously before leaving to stand overnight in the dark.

The sample was filtered under vacuum suction through a filter paper (Whatmans 7cm GFA) using a Hartley 3 piece funnel with Teflon support disk into a clean soveril tube and the filtrate reduced to a volume of approximately 8 ml in a 60°C water bath under oxygen free nitrogen. The sample was then transferred into a 10 ml volumetric flask and made up to mark with chloroform before pouring into clean tubes.

Samples of 0.3 ml were then transferred into 15 ml saponification tubes and 100µl of internal standard (2.6.1a), before drying the solvent in a 60°C water bath under oxygen free nitrogen. Upon drying 2 ml of 2M saponification mixture was added and the samples saponified for 1 hour in a 60°C water bath. The fatty acids were then extracted as described for muscle in section 2.6.4.2 and methylated as below.

2.6.6 Methylation of fatty acids

Frozen samples of feed, digesta, faecal, muscle and adipose tissue fat were taken to dryness in a 60°C water bath under oxygen free nitrogen and 1 ml petroleum spirit added. Diazomethane was added to the sample until the solution turned yellow in colour (10-15 drops). After a quick shake the sample was left to stand in the fume cupboard for 10 minutes before drying in a water bath and blowing down with oxygen free nitrogen. Petroleum spirit was then added (1.5 ml) and samples centrifuged for 4 minutes at 1000 g before transferring into vials ready for gas chromatography.

2.7.7 Gas-Liquid Chromatography

Fatty acids were analysed by gas liquid chromatography as described by Whittington *et al.* (1986). The column was a 50 m x 0.25 mm i.d x 0.39 mm o.d x 0.2 µm film thickness, wall coated open tubular (WCOT) fused Silica capillary column, with CP-Silica 88 for fatty acid methyl esters (FAME) as the stationary phase (Chrompak Ltd., Cat. 7488). The chromatograph was a Carlo Erba Strumentazione HRGC 5160 Mega Series with an A200S

autosampler with flame ionisation detection and helium as the carrier gas split 70:1. Samples of 0.5 µl in petroleum spirit were injected at 214°C. Column temperature was 181°C and increased at 1.5°C per minute up to 220°C where it was held until the end (*ca.* 45 minutes when C22:6 fatty acid was eluted).

Fatty acids were quantified using an Infotronic intergrater (Carlo Erba Merga Series) and C21-heneicosanoic acid methyl esters (Sigma Chemical Co. Ltd Poole) added prior to saponification as an internal standard. Each major peak was identified by comparison of retention times with those of known standards. Linearity of response was confirmed using a GLC-50 monoenoic reference mixture (Supelco, Poole Dorset) consisting of C16:1, C18:1, C20:1, C22:1 and C24:1 in equal quantities (20 % each).

2.6.7.1 Fatty acid (FA) quantification from chromatograms

Recalculating % composition by removing the standard (C21);

$$\text{True \% fatty acid} = \left[\frac{(\text{Fatty acid \% in data} \times 100)}{(100 - \text{\% C21 in data})}\right]$$

Calculating fatty acids mg in 100 g of muscle using the internal standard weight and weight of sample analysed (feed, digesta, faecal, adipose tissue or muscle);

$$\text{Wt of total fatty acids (mg/100 g muscle)} = \left[\left(\frac{100}{\text{\% C21}}\right) \times \left(\frac{\text{Weight C21 (mg)}}{\text{Weight of sample}}\right)\right] \times 100$$

Therefore:

$$\text{Weight of FA (mg/100 g muscle)} = \left[\frac{\text{Total fatty acid Weight (mg/100 g)} \times \text{True fatty acid \%}}{100}\right]$$

2.7 DETERMINATION OF PLASMA AND MUSCLE VITAMIN E

Plasma α-tocopherol was analysed using a high performance liquid chromatography (HPLC) according to the method of Burton *et al.* (1985). Muscle samples were analysed using a modified HPLC procedure of Arnold *et al.* (1993). Samples and working standards were protected from direct sunlight and fluorescent light by extracting under dimmed natural light and using amber-stained glassware and vials. All solvents used were HPLC grade (Rathburn Chemicals Ltd).

2.7.1 Plasma α-tocopherol saponification and extraction

Plasma sample was thawed and mixed thoroughly before transferring 1 ml sample into a 15 ml soveril tube. To the sample was added 2 ml ethanol and the sample vortexed for 15 seconds to precipitate the protein. The sample was then saponified using 1 ml of a 0.1M sodium dodecyl sulphate (SDS $C_{12}H_{25}O_4SNa$) solution and the fat extracted using 1 ml *n*-hexane with 0.05 % BHT as an antioxidant. The sample was vortexed for 60 seconds and centrifuged for 2 minutes at 1000 *g* to clarify the solvent layers. The clear supernatant was transferred using a Pasteur pipette into a clean Soveril tube. The extraction procedure was repeated twice with 1 ml hexane in each extraction.

The samples was dried under oxygen free nitrogen in a 60°C water bath. The residue was redissolved in 1 ml hexane and then transferred into amber coloured HPLC vials. Samples were stored at minus 20°C ready for HPLC reading. Recovery tests were carried out after appropriate dilutions of α-tocopherol (Sigma T-3251) with hexane and addition of known concentrations to plasma samples as presented in Table 3.

Table 2.3 *Plasma α-tocopherol recovery (n = 3)*

α-tocopherol added (μg)	Plasma added (ml)	α-tocopherol added (μg/ml)	% Recovery
-	1	1.38	-
4.13	-	4.07	98.5
1.37	1	2.77	100.7
4.13	1	5.55	101.8
6.88	1	8.36	101.2
		Mean	101±2

2.7.2 Muscle α-tocopherol saponification and extraction

Vacuum packed frozen *Longissimus dorsi* muscle samples were partially thawed and trimmed off all visible fat and connective tissue before chopping and blending using a mini food processor. Approximately 15 g muscle samples was weighed in duplicate and transferred into 500 ml round bottomed quick fit flasks containing 80 g of 40 % glycerol in ethanol (800 ml glycerol in 1200 ml ethanol) and 1 g ascorbic acid (Sigma).

Recovery tests was carried out after appropriate dilutions of α -tocopherol (Sigma T-3251) with hexane and addition of 20 μg solutions at this stage. Using a Polytron mixer the sample was homogenised at 20 000 rpm and then 40 ml ethanol and 40 ml saponification mixture (500 g potassium hydroxide pellets in 100 ml distilled water) added and saponification carried out under reflux for 35 minutes at 95°C before cooling the sample flasks on ice.

The saponified sample was then transferred into 500 ml amber separator flasks containing 100 ml distilled water and 20 ml ethanol. The flask was washed with 100 ml hexane and the washing added to the separator contents and the extract vigorously shaken for 30 seconds. After separation of layers the supernatant was transferred into a second separating flask containing 100 ml of distilled water. The aqueous phase was further extracted twice with hexane before discarding. The bulked hexane extracts were washed with 100 ml distilled water three times or until free of any alkali (tested using a litmus paper). The washed hexane extract was then passed through a short column of anhydrous sodium sulphate into a clean flask containing anhydrous sodium sulphate.

The samples was then evaporated to dryness using a vacuum rotor evaporator on a water bath at 60°C. The residue was redissolved in hexane and transferred into a 10 ml volumetric flask and made up with hexane before mixing and filtering through 0.2 μm pore size (Whatman PVDF syringe filters) into amber HPLC vials and storing at minus 18°C ready for the HPLC.

2.7.3 High Performance Liquid Chromatography (HPLC)

The HPLC system comprised of two Gilson pumps, a Krontron 360 autosampler, a two channel 980 programmable fluorescence detector (Applied Biosystems) and an integrator (Kontron data system 450-MT2).

The system was fitted with an injector valve consisting of a 100 µl loop and a normal phase analytical column of 4.6 mm i.d x 250 mm length, packed with Techsphere silica 5 µ (hplc-Technology). The mobile phase consisted of 96 % hexane and 4 % 1,4 dioxane pumped isocratically at 1.6 ml per minute. The column was insulated and the mobile phase held in a 30°C water bath to avoid fluctuations in column temperature. Fluorescence detection was at an excitation of 297nm and emission of 330nm with a run time of 8 minutes (0-320 seconds). Retention time for α-tocopherol was on average between 5-6 minutes.

2.7.3.1 Calibrations and quantification

Stock solutions of 10 % α-tocopherol (95 % purity Sigma) were prepared in hexane and stored in amber vials at minus 18°C. Appropriate standard dilutions were made before use with hexane and concentrations calculated using the standard absorbency or extinction coefficient (E^0 = 71 for 1 % solution in 1 cm cell at 294 nm wavelength). Quantification was based on an external standard method calibrations ranging from 0 for hexane 1, 3 and 5 µg/ ml. Injections were carried out in duplicate and the column recalibrated after each sample. Sample and standard peaks were then electronically calculated.

Alpha tocopherol content was calculated as below;

$$\text{Plasma } \alpha\text{-tocopherol } (\mu g/ml) = \left[\frac{\text{Peak height of sample x } \mu g \text{ / ml standard}}{\text{Peak height of standard}}\right]$$

$$\text{Muscle } \alpha\text{ -tocopherol } (\mu g/ g) = \left[\frac{\text{Peak height of sample x ug / ml standard}}{\text{Peak height of standard}}\right] \times \left[\frac{10}{\text{Sample weight (g)}}\right]$$

CHAPTER 3

RUMEN BIOHYDROGENATION OF POLYUNSATURATED FATTY ACIDS AND THEIR EFFECTS ON MICROBIAL EFFICIENCY AND NUTRIENT DIGESTIBILITY IN SHEEP

3.1 INTRODUCTION

Dietary lipids upon ingestion are extensively hydrolysed and unsaturated fatty acids biohydrogenated by rumen microbes to more saturated end products, such as stearic acid and *trans* fatty acids (Harfoot and Hazlewood, 1988). Additionally, lipids added to ruminant diets in excess of 5 % often depress ruminal fermentation and fibre digestion (Sutton *et al.* 1983), but the degree of inhibition varies with the fat source and the basal diet (Doreau *et al.* 1991). Feeding whole oilseeds may provide a natural seed coat protection to lipids against rumen biohydrogenation (Scott and Ashes, 1975), and also protect rumen microbes from the toxic effects of lipids in the rumen. Long chain PUFA, especially those from fish oil have been reported to resist biohydrogenation *in vitro* (Ashes *et al.* 1992b), whilst other reports showed that these fatty acids may be extensively biohydrogenated (Doreau and Chilliard, 1997).

The objectives of the present study were to quantify the extent to which dietary polyunsaturated fatty acids from whole linseed and fish oil were biohydrogenated in the rumen, their into the duodenum and to determine their effects on microbial efficiency and whole tract nutrient digestibility.

3.2 MATERIALS AND METHODS

3.2.1. Experimental animals and housing

Four wether sheep aged 9 months, with an average live weight of 55 kg, fitted with permanent rumen cannula and a 'T'-piece cannula in the proximal duodenum were used in a 4 x 4 Latin square design. Each period lasted for 28 days (Figure 3.1), with 14 days adaptation to the diets prior to sample collection. Animals were housed in individual sawdust bedded pens between periods and transferred to metabolism crates during the collection period. The building was continuously illuminated and animals had free access to fresh water.

3.2.2 Diets and feeding procedure

Four iso-energetic and iso-nitrogenous diets based on dried grass were formulated to provide similar fat levels from different sources (Table 3.1). 1: Control (Megalac) diet contained 44 g/kg Megalac (a calcium soap of palm oil; Volac Ltd., Royston); 2: Linseed diet, 105 g/kg whole linseed; 3: Fish oil diet, 36 g/kg fish oil and 4: Linfish diet had a mixture of 52 g/kg whole linseed+18 g/kg fish oil (50:50 on an oil weight basis). The fish oil was South American herring containing a minimum 30 % PUFA and 500 ppm BHT as an antioxidant (Issac Spencer and Co. Fleetwood Ltd., Lancashire). Diets were fed as pellets measuring 10 mm in diameter and 25 mm in length. During each collection period animals were fed using automatic feeders at the rate of 1.2 kg fresh weight per day in twelve equal portion at two hourly intervals. All animals had an initial 3 weeks adaptation period to the diets before commencing the experiment.

3.2.3 Experimental procedure

Dry matter flow at the duodenum was estimated by the dual phase marker technique (Faichney, 1975) using ytterbium acetate and chromium ethylene diamine tetra-acetic acid (Cr-EDTA) as the solid and liquid phase markers respectively as described in sections 2.2, 2.2.4 and 2.2.5 of materials and methods. Commencing on day one of marker infusion, a total faecal collection was carried out using faecal harnesses for 7 days (Figure 3.1). Total faeces voided daily was weighed and thoroughly mixed, after which a 10 % sample was taken and frozen at minus 20°C prior to subsequent analysis. After 8 days of marker infusion duodenal samples were collected as described in section 2.2.3 after which samples of rumen fluid were taken and rumen microbes isolated as described in sections 2.2.2 and 2.2.2.1 of material and methods.

Table 3.1 *Raw materials and chemical composition of diets containing different fat sources*

	Megalac	Linseed	Fish oil	Linfish
Ingredients (g/kg)				
Dried grass	758	735	769	753
Sugarbeet pulp (molassed)	105	105	105	105
Megalac	44	-	-	-
Whole linseed	-	105	-	52
Fish oil	-	-	36	18
Soybean meal	58	20	55	38
Molasses	25	25	25	25
Mineral and Vitamin premix	20	20	20	20
Ammonium chloride	5	5	5	5
Salt	5	5	5	5
Chemical composition (g/kg DM)				
Dry matter	927	929	928	929
Organic matter	912	921	912	920
Crude protein	139	143	139	141
Neutral detergent fibre	477	476	486	479
Total fat	47.8	48.0	47.6	47.2
Vit E IU/kg (α-tocopherol acetate)	183.1	212.3	181.5	196.2
GE MJ / kg DM	20.47	21.08	20.93	21.07

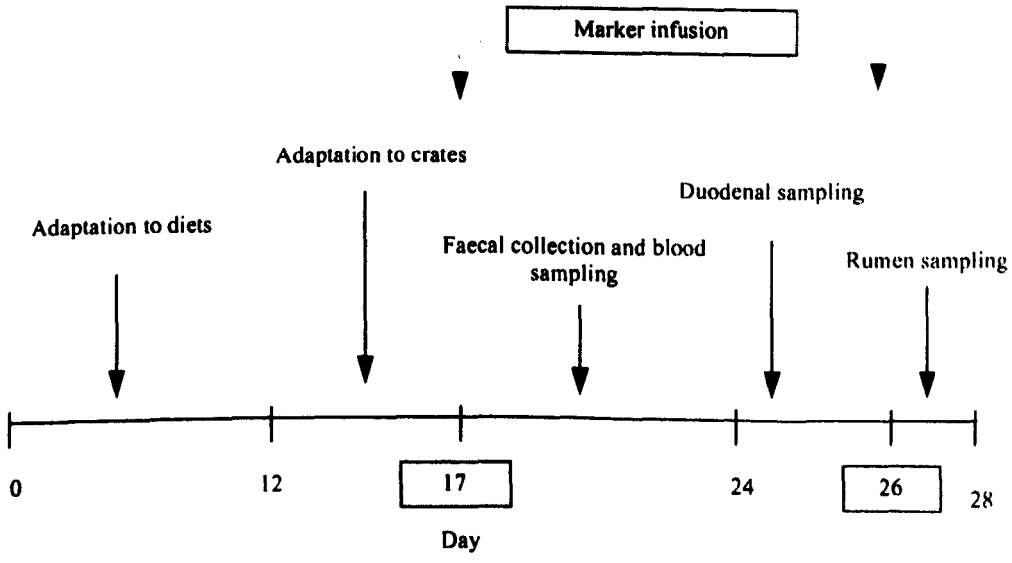


Figure 3.1 Experimental procedure

3.2.4 Chemical analysis

Feed, pooled faecal samples and digesta samples were analysed for dry matter, ash, total nitrogen, ammonia nitrogen and neutral detergent fibre as described in sections 2.1 of materials and methods. Feed and faecal samples were also analysed for gross energy using a bomb calorimeter. Chromium and ytterbium concentrations of the infusion solutions and duodenal samples were determined by atomic absorption spectrophotometry (Siddons *et al.*, 1985) as described in sections 2.2.4. Volatile fatty acids were determined by the method of Ryan (1980), and purine content of the isolated rumen microbes and duodenal digesta was determined using the method of Zinn and Owen (1986) as described in sections 2.4 and 2.5. Fatty acids in feed, faeces and duodenal digesta were extracted by direct saponification and methylated using diazomethane as described in sections 2.6.2 and 2.6.3. Vitamin E in feed was analysed by Roche Vitamin and Chemical Control Laboratory (Hertfordshire).

3.2.5 Calculations and statistical analysis

Digesta flows and apparent nutrient digestibility in the rumen were calculated after mathematical reconstitution of true digesta as described in sections 2.2.5 after Faichney (1975) and microbial efficiency calculated as described in sections 2.5.3. Apparent individual fatty acid digestibility was calculated between the proximal duodenum and rectum and apparent whole tract nutrient digestibility from the mouth to the rectum. Ruminal biohydrogenation was calculated from the changes in the proportion of individual fatty (e.g. C18:0, C18:1, C18:2*n*-6 and C18:3*n*-3) in the duodenal digesta compared with the original proportion in the feed (Wu *et al.* 1991), under the assumption that changes in proportions of these fatty acids in the rumen due to other factors other than biohydrogenation were not significant (Wu and Palmquist, 1991).

$$\text{Biohydrogenation (\%)} = 100 - 100 \times \left[\frac{\left(\frac{\text{Individual unsaturated FA}}{\text{Total FA}} \right) \text{in duodenal digesta}}{\left(\frac{\text{Individual unsaturated FA}}{\text{Total FA}} \right) \text{in diet}} \right]$$

Where all fatty acids (FA) in mg/kg DM

Results were analysed by analysis of variance based on a 4x4 Latin square. All statistical analysis was performed using Genstat 5, (Lawes Agricultural Trust, 1995).

3.1 RESULTS

3.3.1 Diet fatty acid composition

All diets had a similar chemical composition (Table 3.1). Fatty acid composition (g/kg DM) and total fatty acid intake (g/d) is presented in Table 3.2. The inclusion of Megalac in the control diet resulted in the highest concentrations of palmitic (C16:0) and oleic (C18:1) acids, whilst the linseed diet had the highest concentrations of α-linolenic acid (C18:3*n*-3). Similarly the inclusion of fish oil resulted in high concentrations of the long chain *n*-3 fatty acids, EPA (C20:5*n*-3) and DHA (C22:6*n*-3). The linfish diet gave levels of C18:3 *n*-3, C20:5 *n*-3 and C22:6 *n*-3 intermediate between the linseed and fish oil diets (Table 3.2). Stearic acid (C18:0) concentrations was low in all four dietary treatments and only traces of C18:1 *trans* were detected in any of the diets.

Table 3.2 *Fatty acid composition and total fatty acid intake for diets containing different fat sources*

	g/kg DM			
Fatty acid	Megalac	Linseed	Fish oil	Linfish
C12:0 lauric	0.3	0.1	0.2	0.1
C14:0 myristic	0.7	0.2	4.1	2.4
C16:0 palmitic	22.4	5.1	9.6	8.5
C16:1 palmitoleic	0.3	0.3	3.3	2.3
C18:0 stearic	2.0	1.9	1.4	1.8
C18:1 *trans*	0.1	-	0.1	_
C18:1 *n*- 9 oleic	10.7	7.3	4.0	7.4
C18:1 *n*-7 vaccenic	0.3	0.4	0.9	0.9
C18:2 *n*-6 linoleic	4.9	6.8	4.1	7.1
C18:3 *n*-3 linolenic	4.5	24.4	6.2	20.0
C20:5 *n*-3 eicosapentaenoic	0.2	0.2	3.9	2.4
C22:6 *n*-3 docosahexaenoic	_	_	1.9	1.2
RFA[1]	1.4	1.3	7.9	5.9
Total FA intake	47.8	48.0	47.6	47.2

RFA[1]-weight of remaining fatty acids

3.3.2 Duodenal flow of fatty acids

Total fatty acid flow at the duodenum (Table 3.3) did not vary significantly between animals offered any of the four diets but instead were similar to dietary fat intake, which was on average 52.4 g/day. However, the fatty acid composition of duodenal digesta was different

from that in the diet. Duodenal flow of C18:0 was highest in lambs offered the linseed diet, and was higher in lambs offered the Megalac (control) diet than in lambs offered the fish oil or linfish rations ($p<0.001$). The flow of *trans* C18:1 (vaccenic acid) was significantly higher in lambs offered the linfish diet when compared to lambs on the Megalac, linseed or fish oil diets ($p<0.01$).

Table 3.3 *Duodenal fatty acid flow of sheep fed diets containing different fat sources*

	g/day					
Fatty acid	Megalac	Linseed	Fish oil	Linfish	sed	sign
C12:0	0.2	0.1	0.2	0.1	0.03	*
C14:0	0.7	0.3	2.9	1.2	0.19	***
C16:0	22.3	5.0	11.2	7.6	0.66	***
C16:1	0.2	0.2	2.5	0.9	0.18	***
C18:0	15.5	22.9	2.4	4.9	1.15	***
C18:1 *trans*	3.7	9.2	6.9	15.0	1.74	**
C18:1 *n*-9	5.1	2.4	1.8	2.2	0.65	***
C18:1 *n*-7	0.2	0.2	0.9	0.6	0.05	***
C18:2 *n*-6	1.1	0.7	0.7	0.6	0.17	ns
C18:3 *n*-3	1.0	2.1	0.9	1.4	0.20	**
C20:5 *n*-3	0.2	0.2	1.0	0.5	0.02	***
C22:6 *n*-3	–	–	0.6	0.2	0.06	***
RFA[1]	3.3	6.8	14.6	12.2	-	-
Total FA flow	54.1	50.1	49.8	46.2	4.24	ns

RFA[1] -weight of remaining fatty acids

Compared to animals offered the control diet, animals on the linseed diet had double the flow of C18:3*n*-3 at the duodenum ($p<0.01$). Animals offered the linfish diet resulted in a daily flow of C18:3*n*-3 lower than that in animals on the linseed diet, but higher than in animals offered the Megalac and fish oil diets. The duodenal flow of long chain *n*-3 fish oil fatty acids (C20:5*n*-3 and C22:6*n*-3) was double in animals offered the fish oil diet compared to animals on the linfish diets ($p<0.001$), whilst there was negligible flow of long chain *n*-3 PUFA in animals fed either the Megalac or linseed diets.

3.3.3 Biohydrogenation of fatty acids

Rumen biohydrogenation of dietary fatty acids is presented in Table 3.4. The biohydrogenation of C18:1*n*-9 was significantly lower in animals offered diets containing fish oil than in animals offered the Megalac or linseed diets ($p<0.001$). The biohydrohgenation of

C18:2*n*-6 and C18:3*n*-3 ranged between 75 and 95 % in all diets but was significantly higher in the linseed and linfish diets compared to Megalac or fish oil diets ($p<0.05$).

In contrast, longer chain *n*-3 fatty acids present in fish oil were less susceptible to rumen biohydrogenation in animals offered the fish oil and linfish diets. The average biohydrogenation of C20:5*n*-3 was approximately 58 %, while that of C22:6*n*-3 was 60 % for the fish oil diet and 79 % for the linfish diet.

Table 3.4 *Biohydrogenation of fatty acids in sheep offered diets containing different fat sources*

	%					
Fatty acid	Megalac	Linseed	Fish oil	Linfish	sed	sign
C18:1 *n*-9	59.8	66.3	31.2	52.5	4.96	***
C18:2 *n*-6	80.7	89.3	79.8	87.1	2.50	*
C18:3 *n*-3	81.6	91.5	82.2	90.1	2.54	*
C20:5 *n*-3	nd[1]	nd	60.1	56.4	1.67	ns
C22:6 *n*-3	nd	nd	60.2	79.0	5.10	ns

nd[1] - not detected

3.3.4 Apparent fatty acid digestibility

The apparent fatty acid digestibility (from proximal duodenum to the rectum) is presented in Table 3.5. When animals were offered the fish oil and linfish diets the apparent digestibility of individual fatty acids, C12:0+C14:0, C16:0 and *trans* C18:1 were higher than in animals offered the control or linseed diets. Similarly, the total apparent intestinal fatty acid digestibility was highest in animals offered diets containing fish oil ($p<0.05$).

The digestibility of C18 fatty acids was numerically greater for acids with one or two double bonds. But, *trans* 11-C18:1 was numerically more digestible than C18:1*n*-9 in animals offered the fish oil and linfish diets. Animals offered the linseed diet had the lowest digestibility of C16:0 ($p<0.01$). The digestibility of C22:6*n*-3 was almost 100 %, while that of C20:5*n*-3 was 74 % when animals were offered the fish oil diet and 40 % when fed the linfish diet ($p<0.05$).

3.3.5 Rumen volatile fatty acids

Rumen pH and volatile fatty acid (VFA) composition is presented in Table 3.6. The mean rumen pH was 6.4 and was similar when animals were offered any of the four dietary

treatments. Animals offered the fish oil diet had significantly increased proportions of propionate ($p<0.05$). The ratio of ketogenic to glucogenic acids [(acetate+butyrate)/propionate] was numerically lower when animals were on the fish oil diet. When animals were offered the linseed diet there was a trend towards an increase in the proportion of isovalerate in the VFA mixture.

Table 3.5 *Apparent fatty acid digestibility coefficients in sheep offered diets containing different fat sources*

Fatty acid	Megalac	Linseed	Fish oil	Linfish	sed	sign
C12+C14	0.19	negative	0.66	0.41	0.16	*
C16:0	0.46	0.32	0.70	0.62	0.07	**
C18:0	0.56	0.39	0.58	0.64	0.08	ns
C18:1 *trans*	0.74	0.68	0.90	0.90	0.07	*
C18:1 *n*-9	0.83	0.75	0.83	0.80	0.05	ns
C18:2 *n*-6	0.77	0.77	0.71	0.63	0.09	ns
C18:3 *n*-3	0.59	0.81	0.63	0.71	0.14	ns
C20:5 *n*-3	negative	negative	0.74	0.40	0.20	**
C22:6 *n*-3	–	–	0.97	1.0	0.01	*
Total fat[1]	0.50	0.45	0.73	0.71	0.07	*

Total fat[1]- Whole tract fat digestibility

Table 3.6 *Ruminal volatile fatty acid concentrations of sheep offered diets containing different fat sources*

	Megalac	Linseed	Fish oil	Linfish	sed	sign
Rumen fluid pH	6.3	6.3	6.5	6.5	0.100	ns
VFA mol/100 mol						
Acetate	68.3	68.2	65.1	67.6	2.15	ns
Propionate	20.9	20.2	24.5	20.6	1.20	*
Butyrate	9.4	9.6	8.2	9.1	0.76	ns
Iso-butyrate	0.09	0.15	0.16	0.51	0.169	ns
Isovalerate	0.19	0.54	0.39	0.54	0.151	ns
Valerate	0.80	0.95	0.87	1.09	0.326	ns
Caproate	0.35	0.38	0.77	0.55	0.328	ns
[1](C2+C4)/C3	3.73	3.86	3.01	3.73	0.257	ns

[1](C2+C4)/C3-(acetate + butyrate) to propionate ratio

3.3.6 Duodenal flow of nitrogenous components and nutrient digestibility

The duodenal flow of nitrogenous components is presented in Table 3.7. Ammonia-nitrogen flow in animals offered the control diet was significantly lower than in animals on either linseed or fish oil containing rations ($p<0.01$). Similarly, animals offered the control ration had the highest flow (g/d) of non-ammonia nitrogen and microbial nitrogen at the duodenum, whilst animals offered diets containing fish oil had a significantly reduced flow of microbial nitrogen compared to animals on either the Megalac or linseed diet ($p<0.05$).

Microbial efficiency and rumen digestibility is presented in Table 3.8. The efficiency of microbial protein synthesis was significantly higher when animals were offered the Megalac diet than when on any of the other three diets, either when expressed as organic matter apparently or truly degraded in the rumen. Microbial efficiency was however similar when animals were offered any of the other three diets. In contrast, the proportion of organic matter truly digested in the rumen was significantly greater when animals were offered the linseed diet than when offered the control, fish oil and linfish diets ($p<0.05$). Rumen fibre digestibility was significantly lower when animals were offered the fish oil diet.

3.3.7 Apparent whole tract nutrient digestibility

Apparent dry matter, organic matter, crude protein and energy digestibility were not significantly different when animals were offered any of the four diets (Table 3.9). However the apparent total fat digestibility was significantly higher when animals were on diets containing fish oil ($p<0.01$). There was a trend towards higher nutrient digestibility when animals were offered diets containing fish oil than when on the Megalac or linseed diets ($p<0.1$).

Table 3.7 *Dietary nitrogen concentration and duodenal nitrogen fractions of sheep offered diets containing different fat sources*

	g/kg DMI					
	Megalac	Linseed	Fish oil	Linfish	sed	sign
Total nitrogen intake	24.64	25.17	24.46	24.82		
Duodenal flow						
Ammonia-nitrogen	0.70	1.36	1.25	1.03	0.136	*
Non-ammonia-nitrogen	22.05	18.44	19.98	17.37	0.715	**
Microbial-nitrogen	19.57	18.66	15.39	13.47	1.948	ns

Table 3.8 *Microbial efficiency, dietary concentration of organic matter and fibre and proportion digested in the rumen of sheep offered diets containing different fat sources*

Fatty acid	Megalac	Linseed	Fish oil	Linfish	sed	sign
Microbial efficiency						
g N/kg OMADR	45.1	36.8	34.2	24.4	4.73	*
g N/kg OMTDR	28.0	23.1	23.6	18.8	1.67	***
Organic matter (g/kg DM)						
Dietary concentration	912	921	912	920		
Truly digested in rumen	687	786	648	708	36.6	*
Proportion digested in rumen	0.734	0.853	0.711	0.769	0.0397	*
NDF (g/kg DM)						
Dietary concentration	477	476	486	479		
Digested in the rumen	272	296	249	292	7.20	***
Proportion digested	0.571	0.622	0.512	0.610	0.0152	***

Table 3.9 *Apparent whole tract digestibility coefficients of sheep offered diets containing different fat sources*

	Megalac	Linseed	Fish oil	Linfish	sed	sign
Dry matter	0.63	0.62	0.66	0.65	0.039	ns
Organic matter	0.65	0.64	0.68	0.67	0.027	ns
Crude protein	0.63	0.63	0.66	0.66	0.042	ns
NDF	0.59	0.58	0.63	0.61	0.045	ns
Total fat	0.49	0.50	0.76	0.80	0.079	*
GE	0.63	0.62	0.66	0.66	0.039	ns

3.4 DISCUSSION

3.4.1 Fatty acid intake and flow to the duodenum

The fatty acid composition of the four dietary treatments reflected the fatty acid composition of the respective fat sources. The Megalac diet was high in C16:0, the linseed diet was high in C18:3*n*-3 and the fish oil diet was high in C20:5*n*-3 and C22:6*n*-3. The linfish diet contained C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 at levels intermediate between the control diet and the linseed and fish oil diet.

Duodenal fatty acid flow (g/day) in the current experiment did not vary significantly when animals were offered any of the four diets and was approximately 103, 95, 95 and 89 % of dietary intake for animals offered the Megalac, linseed, fish oil and linfish diets. Negative as well as positive fatty acid balances across the rumen have previously been reported and are attributed to variability in microbial *de novo* fatty acid synthesis in the rumen (Doreau and Chilliard, 1997; Wu *et al.* 1991; Zinn, 1988 & 1989; Bock *et al.* 1991). The microbial fatty acid contribution to duodenal digesta is estimated to be 10-15 g/kg organic matter digested in the rumen in dairy cattle (Wu *et al.* 1991). However the regression of duodenal fat flow against dietary fat intake gives a gradient of 0.92, indicating losses of dietary fat in the rumen (Jenkins, 1993). This observation may be associated with an earlier demonstration that rumen microbes were capable of reducing their rate of *de novo* fat synthesis when in the presence of high amounts of exogenous fat (Demeyer *et al.* 1978). This suggests a preferential utilisation of dietary lipids, which is energetically more efficient than microbial *de novo* fat synthesis (Demeyer and Van Nevel, 1995).

The observed non-significant differences between dietary fat intake and duodenal fat flow, suggests that microbial *de novo* fatty acid synthesis did not vary when animals were offered any of the four diets. However, the positive balance of duodenal fat flow when animals were offered the control diet suggests a higher contribution of microbial *de novo* fat synthesis to duodenal fat flow than when animals were on diets containing *n*-3 PUFA sources. As will be discussed later dietary PUFA have been shown to be toxic to rumen microbes and have consistently been associated with rumen defaunation as well as decreased microbial efficiency (Ikwuegbu and Sutton, 1982 and Broudiscou *et al.* 1994).

Other factors such as fatty acid metabolism in the rumen (Jesse *et al.* 1992) or an underestimation of duodenal flow as suggested by Murphy *et al.* (1987) have all been cited as possible explanations for the variability between dietary fat intake and duodenal fat flow. In a review of the literature, Doreau and Ferlay (1994) reported that the microbial contribution to duodenal fatty acid flow was related by the equation: FAD = 0.801 FAI + 9.29, where FAD is the fatty acid flow at the duodenum (g/kg DMI) and FAI is the fatty acid intake (g/kg DM) a relationship which was independent of fat source or method of protection. Using this equation it can be predicted that dietary fat levels below 50 g/kg DM will result in positive duodenal fatty acid balances whilst those greater than 50 g/kg DM will give negative balances. The current finding that a dietary fat level of 50 g/kg DM resulted in a balance between intake and duodenal flow is therefore consistent with these results.

3.4.2 Rumen biohydrogenation

The higher duodenal flow of C18:0 compared to intake reflects ruminal biohydrogenation of C18 unsaturated fatty acids in all the diets. In the control diet C18:1*n*-9 in Megalac, a calcium protected soap partially resisted biohydrogenation. This low biohydrogenation of C18:1*n*-9 in Megalac may have resulted from a limited availability of free fatty acids in the rumen. Hawke and Silcock, (1969) noted that a free carboxyl group was a pre-requisite for rumen biohydrogenation, hence the partial resistance of Megalac to biohydrogenation may have resulted from incomplete dissociation of the soap in the rumen. Sukhija and Palmiquit, (1990) demonstrated a 50 % dissociation of calcium soap at pH 4.6, which is well below the rumen pH of 6.3 measured in animals offered the Megalac (control) diet in the current study.

Wu *et al.* (1991) observed that biohydrogenation of C18:1*n*-9 was low at low dietary concentrations. This is similar to the findings in the current study where the level of biohydrogenation of C18:1*n*-9 varied from 60-66 % when animals were offered the Megalac or linseed diets, to 31 % when animals were offered the fish oil diet. Rumen biohydrogenation of C18:2*n*-6 was lower at low dietary concentrations, an observation attributed to the preferential incorporation of C18:2*n*-6 into cell vacuoles (Bauchart *et al.* 1990). This incorporation provides C18:2*n*-6 with protection against rumen biohydrogenation which is proportional to dietary C18:2*n*-6 concentration and has been observed even with dietary protected fats (Klusmeyer *et al.* 1991). Despite this preferential protection the biohydrogenation of C18:2*n*-6

was similar to that of C18:3*n*-3 as reported in the literature by Doreau and Ferlay (1994). Harfoot *et al.* (1974) observed that bacteria grown in culture adsorbed more than 90 % of added fatty acids until feed particles were added, then 60 % of the fatty acids become associated with the feed particles. This physical coating effect of lipids onto feed particles, especially at low fatty acid concentration may lower biohydrogenation, because adsorption enhances the outflow of fatty acids from the rumen.

The extent of C18:3*n*-3 biohydrogenation when animals were offered any of the diets was similar to that reported in other studies ranging between 85-100 % (Wu *et al.* 1991 and Murphy *et al.* 1987). However, when animals were offered the Megalac and fish oil diets, the biohydrogenation of C18:3*n*-3 was significantly lower than in animals offered the linseed and linfish diets. In a review of the literature, Doreau and Ferlay, (1994), found no relationship between the dietary concentration of individual fatty acids and the level of rumen biohydrogenation. However, in dried grass (the basal diet in the current work) C18:3*n*-3 was present in the form of glycolipids which are less susceptible to rumen hydrolysis and consequently biohydrogenation by virtue of their location within the cell structure (Noble *et al.* 1984). This may explain the lower rumen biohydrogenation of C18:3*n*-3 when animals were offered the Megalac or fish oil diets, where the majority of C18:3*n*-3 was derived from dried grass.

Increases in tissue unsaturates when ruminant animals were offered diets containing oilseeds instead of oils has been associated with a natural partial protection of unsaturated fatty acids against biohydrogenation (St. John *et al.* 1987; Solomon *et al.* 1991). In the current study the extensive biohydrogenation observed suggests that an intact seed coat conferred little or no protection to C18:3*n*-3 against biohydrogenation. This lack of protection may be due to a disruption of the seed coat during the manufacturing processes e.g. pelleting or during mastication upon ingestion by the animal. However, the inclusion of whole linseed in the diet did result in a doubling of the flow of C18:3*n*-3 at the duodenum in animals offered the linseed diet compared to animals on the Megalac diet.

The long chain fatty acids present in fish oil, C20:5*n*-3 and C22:6*n*-3 were less susceptible to rumen biohydrogenation than C18:3*n*-3, a finding similar to that suggested by Ashes *et al.* (1992b) who incubated fish oil casein *in vitro* with rumen contents from sheep. Ashes *et al.*

(1992b) observed that the inability of rumen microorganisms to hydrogenate fish oil fatty acids compared to C18 fatty acids was not a result of fish oil inhibiting lipase activity in the rumen, because the degree of lipolysis was similar for fish oil and sunflower oil. Instead the authors suggested that rumen micoorganisms lacked the specific enzymes or steric factors necessary to biohydrogenate fish oil fatty acids. In the current study the differences between the extent of C18:3*n*-3 and fish oil fatty acids biohydrogenation may also be associated with the physical-chemical nature of the substrate. Harfoot *et al.* (1975) observed that fatty acids in the rumen adhere to either feed particles or microbial cells thus impending the passage of essential nutrients required for microbial growth. The extent to which the physical coating occurs probably determines the magnitude of rumen biohydrogenation. Fish oil fatty acids were more unsaturated, more hydrophobic and consequently more toxic to rumen biohydrogenators compared to C18 fatty acids.

The inhibitory effects of PUFA on rumen biohydrogenators was reflected in the concentration of *trans* fatty acids in duodenal digesta. When animals were offered diets with added PUFA there was a significantly higher flow of *trans* C18:1 compared to control fed animals, a result often associated with incomplete biohydrogenation (Abaza *et al.* 1975; Broudiscou *et al.* 1990). The highest concentration of *trans* C18:1 was however recorded when animals were offered the linfish diet reflecting additional inhibitory effects of fish oil on rumen biohydrogenators.

Other factors such as elimination of rumen protozoa by dietary PUFA may have contributed to a decrease in the efficiency of biohydrogenation (Jouany *et al.* 1988). However, Doreau and Chilliard, (1997), recently reported extensive biohydrogenation of the longer chain PUFA present in fish oil in dairy cattle, whilst Choi *et al.* (1997) observed that over 90 % of C20:5*n*-3 and C22:6*n*-3 were biohydrogenated in beef cattle. The low susceptibility of fish oil fatty acids to biohydrogenation in sheep in the current study and in the *in vitro* work of Ashes *et al.* (1992b), suggests a species variation. However, other factors such the effects of the basal diet on rumen metabolism cannot be ruled out and further investigations are required.

3.4.3 Fatty acid digestibility

There are as yet no clear effects of fatty acid composition on digestibility in ruminants, although this issue is often confounded by experiments quoting the fatty acid intake rather than the duodenal fatty acid flow. It is hypothesised that micelle formation may vary between fatty

acids (faster with unsaturated compared to saturated) or that bile production depends on the nature of fat (Doreau and Chilliard, 1997). In monogastrics, unsaturated fatty acids are more digestible than saturated ones of the same lengths as they readily form micelles, which aid in intestinal absorption (Sklan *et al.* 1985; Powles *et al.* 1993). To test this hypothesis in ruminants, Doreau *et al.* (1992), replaced rapeseed oil with hydrogenated fish oil and observed decreased fatty acid digestibility. Thus the higher apparent total and individual fatty acid intestinal digestibility (proximal duodenum to rectum) when animals were offered diets containing fish oil in the current work may be associated with the higher concentration of unsaturated C20, C22 and *trans* C18:1 fatty acids present at the duodenum. Overall individual fatty acid digestibility increased with increasing degree of unsaturation and concentration post ruminally.

The use of faecal fatty acid content to measure fatty acid digestibility has its limitations. Microbial biohydrogenation and *de novo* fatty acid synthesis in the hindgut has been associated with negative fatty acid digestibility (Andrews and Lewis, 1970a & b; Sharma *et al.* 1978). Rumen bacteria have also been shown to preferentially incorporate C18:2*n*-6 compared to other free fatty acids into cell vacuoles (Bauchart *et al.* 1990), which may also occur in the hindgut. This probably explains the very low and negative digestibility observed as well as the low variability in C18:2*n*-6 digestibility across the diets. The low digestibility of C16:0 and C18:0 across the diets (mean of 53 and 54 %, respectively) is similar to that reported by Wu *et al.* (1991) but lower than reported in the review by Doreau and Ferlay, (1994), (79 and 77 % for C16:0 and C18:0, respectively). This observation may be attributed to increased endogenous contribution from bile secretions for C16:0, whilst for C18:0 hind gut biohydrogenation of unsaturated C18 fatty acids (Jenkins, 1993) may have had an important contribution. In addition, fat supplementation can result in a shift in organic matter digestion from the rumen to the hind gut thus increasing microbial fatty acid synthesis (Demeyer, 1991). However, variable fatty acid digestibility both within and between experiments, ranging between 52 to 92 % have been reported in different studies (Doreau and Ferlay, 1994).

3.4.5 Ruminal volatile fatty acids

In common with other studies, rumen pH was not affected by the dietary fat source but the supply of fish oil significantly increased the proportion of propionate (Ikwuegbu and Sutton, 1982; Jenkins, 1993; Doreau and Chilliard, 1997). This result is thought to be due to

the modification of the ruminal microbial ecosystem by dietary PUFA and results in an inhibition of cellulolytic and methanogenic bacteria (Henderson, 1975). The increase in propionate could also be due to a competition for metabolic hydrogen between methane and propionate production pathways as well as the fermentation of glycerol liberated during lipolysis (Demeyer and Van Nevel, 1995; Doreau and Ferlay, 1995).

3.4.6 Microbial protein synthesis, organic matter and fibre digestibility

Duodenal ammonia nitrogen is an index for microbial protein synthesis since it reflects the capture of nitrogen released during deamination of rumen degradable protein. Compared to diets with added PUFA, the control diet resulted in lower concentrations of ammonia nitrogen mainly because of a more efficient continuous recycling of nitrogen normally associated with rumen protozoa (Leng, 1982). Dietary PUFA have been associated with the disappearance of ciliate protozoa from the rumen (Czerkwaski *et al.* 1975; Ikwuegbu and Sutton, 1982; Broudiscou *et al.* 1994). This defaunation decreases the intra-ruminal recycling of nitrogen and consequently increases the efficiency of microbial synthesis, especially when animals are offered a readily fermentable carbohydrate (Jouany *et al.* 1977 and Sutton *et al.* 1983). But when animals are on a diet rich in cell wall carbohydrate defaunation decreases bacterial cellulolytic activity (Jouany and Senaud, 1982), which maybe due to a decrease in rumen organic matter fermentation in the absence of protozoa (Jouany *et al.* 1988).

Sutton *et al.* (1975), reported little effect of 20 g per day fish oil on fibre or organic matter digestion or microbial protein synthesis although this value was considerably lower than the 40 g fish oil per day used in the current work. In beef cattle, Choi *et al.* (1997) reported a non-significant trend towards an increase in fibre digestion in the rumen with the addition of fish oil and similar findings have been reported in dairy cattle by Doreau and Chilliard, (1997). The inclusion of fish oil in the current trial was associated with a significant reduction in the efficiency of microbial protein synthesis when expressed in terms of organic matter apparently or truly degraded in the rumen. This effect could be attributed to a decrease in microbial protein synthesis, rather than a reduction in organic matter digestibility in the rumen, because the latter was not different between the control and fish oil diet.

Doreau and Ferlay (1995), reported that the efficiency of microbial protein synthesis was increased by the addition of saturated and mono-unsaturated fatty acids but not by PUFA

although this effect was only significant when the addition of oil reduced organic matter digestion by between 10-30 %. As earlier discussed the net flow of microbial protein to the duodenum is a product of microbial nitrogen yield and the amount of organic matter fermented in the rumen. If defaunation inhibits the latter more than it increases the former, net bacterial nitrogen flow to the duodenum may be decreased as discussed elsewhere (Demeyer *et al.* 1986). In the current experiment, compared with the control ration, organic matter digestion in the rumen was significantly increased with linseed but not fish oil inclusion.

The effects of added fat on microbial growth are as yet unclear. The addition of lipids *in vitro* has been demonstrated to have a negative effect on microbial growth, this effect being more pronounced for polyunsaturated than saturated fatty acids and is especially marked on cellulolytic bacteria (Doreau and Chilliard, 1997). In a review of the literature published on work conducted *in vivo,* Doreau and Ferlay (1995) reported little effect of supplemental fatty acids, either saturated or unsaturated, on the flow of non-ammonia nitrogen or microbial nitrogen at the duodenum. However, several reports have indicated a beneficial effect of PUFA on microbial protein synthesis (Sutton *et al.* 1983; Broudiscou *et al.* 1994) as well as a negative effect (Czerkawski *et al.* 1975). However, relatively few studies have examined the effects of fish oil on microbial metabolism and digestion.

In the current experiment the form of dietary PUFA appeared to be important with diets containing fish oil reducing both the duodenal flow of microbial nitrogen and the efficiency of microbial protein synthesis compared to the linseed or Megalac diets. Rapid and extensive biohydrogenation of C18:3*n*-3 from linseed has been suggested to reduce the potential antimicrobial effects of this acid in the rumen (Hogan *et al.* 1987). Hence the reduced biohydrogenation of fish oil fatty acids may have had a greater toxic effect on rumen metabolism. This may also explain the reduced microbial protein synthesis observed in the linfish diet possibly because of the additive antimicrobial effect of C18:3*n*-3 from linseed and fish oil fatty acids.

3.4.7 Apparent rumen nutrient digestibility

The negative effects of PUFA on fibre digestion in the rumen in the current study confirm those of previous studies where feeding free oils reduced rumen organic matter and fibre digestion (Sutton *et al.* 1983; Jenkins, 1993; Broudiscou *et al.* 1994). Devendra and

Lewis (1974) summarised four theories to explain these effects. First that the physical coating of fibre by oil impends the passage of nutrients and attachment of cellulase to cellulose. Secondly that the reduced cation availability, from formation of insoluble complexes with long chain fatty acids, reduces microbial growth. Thirdly, the inhibition of microbial activity modifies microbial population. The last hypothesis has been supported by results from several studies where a sharp decrease in protozoal numbers following introduction of PUFA into the diet of sheep has been reported (Ikwuegbu and Sutton, 1982; Sutton *et al.* 1983; Broudiscou *et al.* 1994). Protozoa are known to be active amylolytic and proteolytic microorganisms and can influence a number of bacterial species in the rumen either positively or negatively depending on the supply of fermentable organic matter (Jouny *et al.* 1988). Effects of rumen defaunation are still a subject of much debate, but in the present study it is possible that defaunation contributed to reduced fibre digestion in the rumen although protozoal numbers were not measured.

3.4.8 Apparent whole tract nutrient digestibility

Generally, the addition of lipids to ruminant diets decreases DM and especially cell wall digestibility, or does not modify them (Palmiquist and Jenkins, 1980 and Jenkins, 1993) as observed in the current work. In some cases, slight increases in digestibility have been observed with different lipids supplements (Mir, 1988 and Elmeddah *et al.* 1991). With hydrogenated fish oil, DM and fibre digestibility was unmodified (Doreau, 1992). Sutton *et al.* (1975) did not observe any variation in organic matter and fibre digestibility and ruminal digestion in sheep fed diets supplemented or not with 20 g/day of cod liver oil. Doreau and Chilliard, (1997), infused fish oil into the rumen or duodenum of dairy cattle and reported significant increases in organic matter, DM and neutral detergent fibre digestibility. This may however be attributed to the decrease in feed intake when fish oil was infused in the rumen (Chilliard *et al.* 1995).

Modifications of the VFA profile (discussed above) suggest a change in the ruminal microbial ecosystem, but contrary to the general trend diets containing fish oil did not result in a lower fibre digestibility. Sutton *et al.* (1983) observed that digestive processes in the hindgut, compensated for the reduction in fibre digestion in the rumen resulting in a limited effect on whole tract fibre digestion. This may explain the numerically greater, but not significant effect

of dietary fish oil on whole tract fibre digestibility despite the low rumen fibre digestibility when animals were offered the fish oil diet.

Whole tract fat digestibility was significantly higher in both diets with fish oil than in the Megalac and linseed diets probably due to the increased flow of unsaturated fatty acid at the duodenum and consequently more efficient absorption. Few other studies have examined the effects of unsaturated fatty acids on fat digestion in ruminants due to the extensive rumen biohydrogenation and the low inclusion rates in ruminant diets. However evidence suggests that unsaturated fatty acids are more digestible than saturated ones of the same length due to their hydrophilic nature which aids micelle formation and digestion (Sklan *et al.* 1985).

3.5 CONCLUSIONS

Dietary C18:3*n*-3 supplemented as whole linseed was extensively biohydrogenated, while fish oil fatty acids, C20:5*n*-3 and C22:6*n*-3 were less susceptible to rumen biohydrogenation. Compared to the control diet (Megalac), feeding diets with whole linseed or fish oil increased the flow of polyunsaturated fatty acids at the duodenum. All PUFA sources irrespective of source decreased rumen microbial protein synthesis and consequently decreased fibre digestion in the rumen. The inclusion of fish oil in the diet increased the proportion of propionate in the rumen volatile fatty acid mixture and whole tract fat digestibility.

CHAPTER 4

EFFECTS OF DIETARY FAT SOURCE AND BREED ON THE FATTY ACID COMPOSITION OF LAMB MUSCLE

4.1 INTRODUCTION

Ruminant products have been criticised for the possible adverse effects of their saturated fatty acids on human health, and consequently their low polyunsaturated to saturated (P:S) ratio attributed to rumen biohydrogenation (Enser *et al.* 1996). However, the *n*-6 to *n*-3 ratio is beneficially lower in pasture compared to concentrate finished animals (Enser *et al.* 1998). This reflects differences in dietary fat composition, grass being high in the *n*-3 series precursor fatty acid α-linolenic acid (C18:3*n*-3) and grains higher in the *n*-6 series precursor fatty acid linoleic acid (C18:2*n*-6).

Few studies have attempted to manipulate the polyunsaturated to saturated fatty acid acid ratio (P:S) and *n*-6 to *n*-3 ratios in lamb which may be controlled by either genetic factors, causing certain breeds or crosses to deposit different levels of polyunsaturated fatty acids, or by the quantity and quality of dietary fat. The previous metabolism study demonstrated that whilst there was extensive biohydrogenation of C18:3*n*-3 from whole linseed, the flow of C18:3*n*-3 at the duodenum of animals offered the linseed diet was doubled compared with animals fed the control (Megalac) diet. The inclusion of fish oil significantly increased the flow of C20:5*n*-3 and C22:6*n*-3 at the duodenum.

This study was designed to investigate the effects of feeding the four different fat sources (Megalac, whole linseed, fish oil and linfish) characterised in the previous study (Chapter 3), on the performance and the intramuscular fatty acid composition of three extreme sheep breeds.

4.2 MATERIALS AND METHODS

4.2.1 Experimental animals, treatments and design

24 Suffolk x Lleyn, 24 Friesland x Lleyn, and 24 Soay ram lambs, with initial live weights of 26, 24 and 12 kg, respectively were used in the study.

Four iso-energetic and iso-nitrogenous dietary treatments were formulated based on dried grass to provide similar fat level (60 g/kg) from different fat sources (Table 4.1), and were the same diets as used in experiment one (Table 3.1).

Table 4.1 *Raw materials and chemical composition of the diets containing different fat sources*

	Megalac	Linseed	Fish oil	LinFish
Ingredient (g/kg)				
Dried grass	758	735	769	753
Sugarbeet pulp (molassed)	105	105	105	105
Megalac	44	-	-	-
Whole linseed	-	105	-	52
Fish oil	-	-	36	18
Soybean meal	58	20	55	38
Molasses	25	25	25	25
Minerals & Vitamin premix	20	20	20	20
Ammonium chloride	5	5	5	5
Salt	5	5	5	5
Chemical composition (g/kg DM)				
Dry matter	893	897	901	896
Organic matter	878	889	885	887
Crude protein	134	138	135	136
Neutral detergent fibre	459	460	471	462
Total fat	59.	52.9	66.9	63.0

The Control diet contained Megalac, (C16:0); the second diet contained whole linseed (C18:3*n*-3) a precursor for the longer chain *n*-3 fatty acid EPA and DHA; the third diet contained fish oil which supplied preformed long chain *n*-3 PUFA, EPA and DHA and the fourth diet contained a mixture of equal quantities of fat from whole linseed and fish oil.

Dietary vitamin E content was approximately 200 IU/kg across all treatments. The diets were compounded and pelleted by Dalgety Feed Company (Camarthen, Wales). Lambs were allocated by breed and live weight to the four dietary treatments in a 3 x 4 factorial experimental design with blocking based on initial live weight.

4.2.2 Procedure and measurements

Lambs were individually housed in raised floor pens and gradually adapted to a mixed diet containing equal quantities on a weight basis of the four diets. After two weeks on the adaptation diet, all lambs were offered their respective dietary treatments. Feed was offered *ad-libitum* and refusals recorded between 0800 and 0900 hours on a Monday, Wednesday and Friday in each week. Feeding levels were adjusted to 115 % of the consumption calculated from the previous refusal weight. Feed samples were taken every fortnight and stored at 4°C prior to subsequent analysis for dry matter, organic matter, crude protein, neutral detergent fibre and total fatty acids as described in section 2.1.1, 2.1.2, 2.1.3.1, 2.1.4 and 2.6.2, respectively of the Material and Methods (Chapter 2).

Weekly live weights were recorded every Thursday between 1400 and 1500 hours. At approximately half the potential mature live weight for each breed, animals were slaughtered and carcasses scored for conformation and fat class as described in section 2.5.2 of Material and Methods (Chapter 2). This was on average 44 kg for the Suffolk and Friesland crosses and 21 kg for the Soay lambs. Post slaughter samples from the *semimembranosus* (SM) and *longissimus dorsi* (LD) muscles and forelimb joint were taken as described in section 2.5.3 of the Materials and Methods (Chapter 2). All samples were vacuum packed and frozen at minus 20°C, prior to fatty acid analysis as described in sections 2.6.4 and the forelimb dissected as described in section 2.5.3 of the Materials and Methods (Chapter 2).

4.2.3 Statistical analysis

Live weight gain for each animal was estimated from regression of live weight on time and the average daily food intake estimated from total food intake for the experimental period for each animal and expressed on a DM basis. The two parameters were expressed on a metabolic live weight ($W^{0.75}$) basis to avoid confounding effects owing to breed differences in mature body weight. The visual scores for carcass fatness and conformation were converted to

numerical values using the MLC carcass fat conformation scheme for statistical analysis (Kempster *et.al.* 1986). All data was subjected to analysis of variance using a factorial randomised block design with the initial live weight being used as a covariant where appropriate (Genstat 5; Lawes Agricultural Trust, 1995).

4.3 RESULTS

4.3.1 Diet composition

Analysed chemical composition (Table 4.1) was similar to diets in Chapter 3 (Table 3.1) but differed in total fat content which was higher in the current study (52-67 g/kg DM) compared to the previous study (*ca.* 49 g/kg DM). The variability in total fat content between diets in the current study may have occurred during the large-scale (commercial) compounding process.

Table 4.2 *Fatty acid composition and total fatty acid intake of the diets containing different fat sources*

	g/kg DM			
Fatty acid	**Megalac**	**Linseed**	**Fish oil**	**Linfish**
C12:0 lauric	0.5	0.2	0.2	0.2
C14:0 myristic	0.7	0.4	3.5	2.0
C16:0 palmitic	22.0	5.5	11.7	8.4
C16:1 *n*-7 palmitoleic	0.2	0.5	3.3	1.8
C18:0 stearic	2.4	1.9	1.9	1.8
C18:1 *trans*	0.1	–	0.1	–
C18:1 *n*-9 oleic	13.3	7.1	7.3	7.8
C18:1 *n*-7 vaccenic	0.5	0.5	1.3	0.8
C18:2 *n*-6 linoleic	11.4	8.9	10.4	10.6
C18:3 *n*-3 α-linolenic	6.6	25.5	8.4	19.5
C20:5 *n*-3 eicosapentaenoic	0.2	0.6	5.7	3.0
C22:6 *n*-3 docosahexaenoic	–	–	3.3	1.6
Unidentified fatty acids	1.4	1.3	7.9	5.9
Total Fat	59.5	52.9	66.9	63.0

Diet fatty acid composition is presented in Table 4.2. The inclusion of Megalac in the control diet resulted in the highest concentration of palmitic (C16:0) and oleic (C18:1*n*-9) acids whilst the inclusion of whole linseed gave highest concentration of α-linolenic acid (C18:3*n*-3). The inclusion of fish oil produced high concentrations of EPA (C20:5*n*-3) and DHA (22:6*n*-3). The combination diet Linfish (whole linseed plus fish oil) had intermediate levels of C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 between the linseed and fish oil diets. Stearic acid (C18:0) was low

while vaccenic acid (*trans* 11-C18:1) concentration was negligible in all diets. Myristic acid (C14:0) concentration was high in diets with fish oil compared to the control and linseed diets.

4.3.2 Diet and breed effects on animal performance

Animal performance data is summarised in Table 4.3. Animals offered both diets containing fish oil had a lower food intake compared to lambs on the Megalac and linseed diets. Mean values for food intake were 132.3, 125.3, 94.1 and 110.4 g/kg $W^{0.75}$ for the Megalac, linseed, fish oil and linfish diets, respectively (sed = 4.30, $p<0.001$). Additionally, compared to the fish oil diet, lambs offered the linfish diet consumed more food (94.1 and 110.4 g/kg $W^{0.75}$, respectively, $p<0.001$) (Table 4.3). Lambs offered the fish oil diet had lower live weight gains than those on the control, linseed or linfish diets. Mean values were 20.8, 20.6, 15.8 and 19.6 g/kg $W^{0.75}$ for lambs offered the Megalac, linseed, fish oil and linfish diets, respectively (sed = 1.35, $p<0.01$). However, dietary fat had no significant effect on feed conversion ratio (FCR, kg feed/kg gain) (Table 4.3).

Daily food intake and daily live weight gains were both highest in Suffolk lambs and lowest in the Soay with the Friesland having intermediate food intake and live weight gain. Mean values: for daily food intake were, 129.2, 98.3 and 129.2 and for live weight gain, 23.1, 15.9 and 18.5 g/kg $W^{0.75}$ for Suffolk, Soay and Friesland lambs, respectively (sed = 3.73 and 1.17, respectively, $p<0.001$). Feed conversion ratio (FCR, kg feed DM/kg gain) was lower in Suffolk lambs than in the Soay or Friesland lambs (5.6, 6.5 and 6.9, respectively, sed = 0.51, $p<0.05$) (Table 4.3).

When fed the Megalac (control) diet, Suffolk lambs ate more than the Friesland, which ate more than the Soay lambs. The mean intake values for Megalac were 152.7, 112.6 and 131.6 g/kg $W^{0.75}$, (sed = 7.46, $p<0.05$) for Suffolk, Soay and Friesland lambs respectively (Table 4.3). Both the Suffolk and Friesland lambs ate more of the linseed diet than the Soay. The mean intake values for the linseed diet were 140.7, 101.3 and 134.1 g/kg $W^{0.75}$ for the Suffolk, Soay and Friesland lambs respectively. Friesland lambs ate more of the fish oil diet than the Soay but not the Suffolk. The mean intake values for the fish oil diet were 96.3, 81.9 and 104.0 g/kg $W^{0.75}$ for the Suffolk, Soay and Friesland lambs respectively. Suffolk lambs also consumed more of the mixed diet than the other two breeds. The mean intake values for the linfish diet

were 127.1, 97.3 and 106.9 g/kg $W^{0.75}$ for the Suffolk, Soay and Friesland lambs respectively (Table 4.3).

4.3.3 Diet and breed effects on carcass weights and classification characteristics

Carcass weights and classification characteristics are summarised in Table 4.3. Lambs fed both diets containing fish oil had higher fat scores than lambs on the Megalac or linseed diets. Mean scores for carcass fat were 11.3, 10.3, 14.2 and 12.2 for the Megalac, linseed, fish oil and linfish diets, respectively (sed = 0.95, $p<0.01$). Compared to lambs on the fish oil diet, lambs offered the linfish diet had a lower fat score (Table 4.3).

Soay lambs had significantly lower hot, cold and forelimb carcass weights than the other two breeds (mean values (kg) for carcass weight were 20.0, 9.7 and 20.0 for hot and 19.3, 9.3 and 19.4 for cold carcasses, sed = 0.31 and 0.30, respectively, $p<0.001$, Table 4.3). The subcutaneous fat weight in the forelimb joint, expressed as a proportion of forelimb weight was significantly lower in the Soay than in the other two breeds, while that of lean (muscle) was lower in Suffolk than in the other two breeds. Mean values (%) for subcutaneous fat were 8.0, 6.1 and 7.5 and for lean 56.1, 58.9 and 57.8 for Suffolk, Soay and Friesland breeds (sed = 0.62 and 0.76, $p<0.01$). Suffolk had a higher proportion of forelimb intermuscular fat than the Soay or Friesland (mean values of intermuscular fat were 11.5, 10.1 and 10.3 %, respectively, sed = 0.58, $p<0.05$, Table 4.3). The carcass conformation score was highest in the Suffolk and lowest in the Soay with the Friesland having an intermediate score (mean conformation scores were 8.8, 3.9 and 7.5, respectively, sed = 0.34, $p<0.01$). There were no significant interactions between diet and breed on carcass weight or classification characteristics.

Table 4.3. *Effects of dietary fat and breed on the performance, carcass weights and classification of sheep*

	Megalac			Linseed			Fish oil			Linfish				Diet	Breed	[1]D x B
	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	SED	sign	sign	sign
Animal performance																
Intake g DM /kg $W^{0.75}$	152.7	112.6	131.6	140.7	101.3	134.1	96.3	81.9	104.0	127.1	97.3	106.9	7.46	***	***	*
DLWG g per kg $W^{0.75}$	25.3	18.9	18.1	25.2	15.2	21.4	18.1	13.0	16.2	24.0	16.5	18.2	2.34	***	***	ns
FCR (feed/gain)	6.13	6.36	8.59	5.64	7.22	6.44	5.40	6.48	6.65	5.34	5.97	6.00	1.027	ns	*	ns
Carcass weights																
Hot Carcass wt. (kg)	19.4	10.1	19.6	20.3	9.9	20.5	19.8	9.0	19.5	20.3	9.6	20.3	0.611	ns	***	ns
Cold Carcass wt (kg)	18.7	9.8	19.0	19.7	9.6	19.2	19.2	8.7	18.9	19.7	9.2	19.6	0.600	ns	***	ns
Carcass pH 24 hours	5.7	5.6	5.7	5.8	5.6	5.7	5.6	5.8	5.8	5.8	5.6	5.6	0.09	ns	ns	ns
Fore limb wt. (g)	1162.8	575.5	1144.0	1212.3	563.8	1192.0	1163.8	521.3	1135.7	1196.7	550.0	1182.2	36.76	ns	***	ns
Bone (%)	24.2	25.1	24.4	24.1	25.8	25.1	24.7	24.9	24.9	24.8	23.9	23.1	1.10	ns	ns	ns
Lean (%)	56.8	58.4	58.5	57.8	59.6	57.8	53.3	59.1	56.0	56.4	58.6	59.0	1.50	ns	**	ns
Subcutaneous fat (%)	7.5	6.3	7.2	7.5	6.1	7.0	9.2	5.8	8.5	7.8	6.2	7.5	1.24	ns	**	ns
Intermuscular fat (%)	11.5	10.2	10.0	10.6	8.6	10.2	12.8	10.2	10.6	11.0	11.3	10.4	1.67	ns	*	ns
Carcass classification																
Conformation score	7.67	4.00	8.00	8.83	4.00	7.67	9.60	3.83	7.33	9.17	3.67	6.83	0.688	ns	***	ns
Fat score	8.50	3.33	8.17	8.50	3.33	6.50	10.60	2.83	9.50	9.50	2.83	8.83	0.550	*	***	ns

[1]D x B = Diet x Breed interaction

4.4 MUSCLE FATTY ACIDS

4.4.1 Fatty acid composition of *semimembranosus* muscle

The weight (mg) of fatty acids per 100 g *semimembranosus* muscle (fatty acid content) is presented in Table 4.4, and the percentage contribution of each fatty acid to total fatty acids (fatty acid composition) is shown in Table 4.5. Total muscle fatty acids ranged between 2.1 to 3.3 % of the tissue weight with no significant differences between treatments or breeds.

4.4.1.1 Main effects of dietary fat and breed on muscle fatty acids

The main effects of dietary fat and breed on muscle fatty acid composition are presented graphically in Figures 4.1 and 4.2, respectively.

(a) *n*-3 fatty acids

The intramuscular lipids of lambs offered the linseed diet contained double the level of C18:3*n*-3 compared with Megalac (control) fed lambs, when expressed on a weight or percentage basis. Mean values were 43.1, 85.6, 38.9 and 55.9 mg/100 g muscle or 1.7, 3.6, 1.5 and 2.2 % for lambs on the Megalac, linseed, fish oil and linfish diets, respectively (sed = 5.14 or 0.16, respectively, $p<0.001$, Table 4.4 and 4.5 and Figure 4.1). The concentration (mg/100 g muscle) of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3, in lambs offered the fish oil diet was more than double that in Megalac (control) fed lambs. Mean values were for C20:5*n*-3 (21.0, 33.7 and 84.5), C22:5*n*-3 (33.7, 20.8 and 23.2) and C22:6*n*-3 (8.5, 13.0 and 26.5) mg/100 g muscle for lambs fed the Megalac, linseed and linfish respectively, (sed = 4.72, 2.28 and 1.46, respectively, $p<0.001$), (Table 4.4). Lambs offered the linfish diet resulted in *n*-3 fatty acid levels intermediate between the linseed and fish oil diets.

Suffolk and Soay lambs contained a higher concentration (mg/100 g) of C18:3*n*-3 than the Friesland lambs. Mean values were 60.3, 59.1 and 49.1 mg/100 g muscle for Suffolk, Soay and Friesland, respectively (sed = 4.50, $p<0.05$, Table 4.4 and Figure 4.2). The contribution of C18:3*n*-3 to the total fatty acids was highest in the Soay lambs than in Suffolk or Friesland lambs (mean values were 2.2, 2.5 and 2.0 % for the Suffolk, Soay and Friesland, respectively, sed = 0.136, $p<0.01$, Table 4.5). The concentration of C20 and C22 *n*-3 PUFA was similar across the three breeds when expressed in mg/100 g muscle, but C20:5*n*-3 and C22:5*n*-3 was higher in Soay than in Suffolk lambs when expressed on a percentage basis. Mean values for

C20:5*n*-3 were 1.6, 2.0 and 2.0 % (sed = 0.18, $p<0.05$) and for C22:5*n*-3 were 1.0, 1.2 and 1.2 % (sed = 0.06, $p<0.05$) for the Suffolk, Soay and Friesland lambs, respectively (Table 4.5).

(b) *n*-6 fatty acids

Lambs on the Megalac diet contained the highest level of C18:2*n*-6, whilst lambs on the linseed diet contained higher levels than those fed either diet with fish oil when expressed on a weight or percentage basis. Mean values were 151.3, 121.7, 102.8 and 104.7 mg/100 g muscle (sed = 7.41, $p<0.001$) or 6.1, 5.2, 4.0 and 4.2 % (sed = 0.40, $p<0.01$) for the Megalac, linseed, fish oil and linfish diets, respectively (Tables 4.4 and 4.5 and Figure 4.1).

When the data was expressed on a weight basis, the concentration of C20:4*n*-6 was highest in lambs offered the Megalac diet, while lambs on the linseed diet contained higher levels than lambs fed either diet containing fish oil. Mean values were 39.3, 31.7, 26.8 and 28.8 mg/100 g muscle for Megalac, linseed, fish oil and linfish diets, respectively (sed = 2.01, $p<0.001$), (Table 4.4 and Figure 4.1). When expressed as a percentage of total fatty acids (Table 4.5), C20:4*n*-6 concentrations was higher in lambs fed the Megalac (control) diet, than in lambs on either diet with fish oil, but was similar to that in lambs fed the linseed diet. Mean values for C20:4*n*-6 were 1.6, 1.4, 1.1 and 1.2 % for Megalac, linseed, fish oil and linfish diets, respectively (sed = 0.18, $p<0.01$).

Soay lambs had higher intramuscular concentrations of all major *n*-6 PUFA than the Suffolk and Friesland, while Friesland contained higher levels of C20:4*n*-6 than the Suffolk when expressed on a weight or percentage basis. Mean values for C18:2*n*-6 were 116.9, 130.9 and 113.2 mg/100 g muscle, (sed = 6.39, $p<0.05$, Table 4.4 and Figure 4.2) or 4.3, 5.5 and 4.9 % (sed = 0.34, $p<0.01$, Table 4.5) for Suffolk, Soay and Friesland lambs, respectively. Mean values for C20:4*n*-6 were 27.2, 35.8 and 32.0 mg/100 g muscle, (sed = 1.91, $p<0.001$, Table 4.4 and Figure 4.2) or 1.0, 1.5 and 1.5 % (sed = 0.16, $p<0.01$, Table 4.5) for Suffolk, Soay and Friesland lambs, respectively.

(c) Monoenoic and saturated fatty acids

The concentration of C18:1*n*-9 (major monoenoic acid) was similar in all lambs on any of the treatments when expressed on a weight basis. However, when expressed as a percentage of total fatty acid, lambs fed the Megalac and linseed diets contained more C18:1*n*-9 than

lambs on the fish oil and linfish diets. Mean values were, 875, 741, 703 and 705 mg/100 g muscle (sed = 79.2) and 33.5, 30.6, 25.0 and 27.5 % (sed = 0.81, $p<0.001$), for Megalac, linseed, fish oil and linfish diets, respectively (Table 4.4 and 4.5). By contrast, lambs offered the fish oil diet had the highest concentration of C18:1*n*-7 and C16:1*n*-7, when expressed on a weight or percentage basis. Mean values for C18:1*n*-7 were 31.8, 26.8, 54.1 and 37.5 mg/100 g muscle (sed = 4.11, $p<0.001$) or 1.3, 1.1, 2.0 and 1.5 % (sed = 0.10, $p<0.001$), and mean values for C16:1*n*-7 were 51.9, 43.0, 69.9 and 53.8 mg/100 g muscle (sed = 6.63, $p<0.01$) or 2.0, 1.8, 2.5 and 2.1 % (sed = 0.08, $p<0.001$) for lambs on the Megalac, linseed, fish oil and linfish diets, respectively (Table 4.4 and 4.5).

Soay lambs contained a smaller proportion of C18:1*n*-9 in the total fatty acids compared to the other two breeds (mean values were 29.8, 27.5 and 30.4 % in Suffolk, Soay and Friesland lambs, respectively sed = 0.70, $p<0.001$), (Table 4.5), but this difference was not apparent when the data was expressed on a weight basis (Table 4.4).

The concentration of C18:0, was highest in lambs offered the Megalac and linseed diets, where it constituted a significantly greater proportion of the total fatty acid with mean values of 13.5, 13.3, 11.3 and 11.4 % for lambs offered the Megalac, linseed, fish oil and linfish diets, respectively (sed = 0.43, $p<0.001$, Table 4.5). Lambs offered the Megalac, fish oil and linfish diets contained a higher concentration of C16:0 than lambs on the linseed diet (mean values were 24.4, 20.7, 24.1 and 22.9 %, respectively for Megalac, linseed, fish oil and linfish diets, respectively, sed = 0.40, $p<0.001$). By contrast, when expressed on a weight basis the content of C18:0 was similar all lambs on any of the four dietary treatments, whilst the content of C16:0 tended to be lower in lambs fed the linseed diet (mean values for C16:0 were 639, 500, 669 and 594 mg/100 g muscle for lambs on the Megalac, linseed, fish oil and linfish diets, respectively, sed = 64.4, $p = 0.057$), (Table 4.4).

Suffolk lambs had higher intramuscular concentrations of C18:0 than either the Friesland or Soay lambs when expressed on a weight or percentage basis (Table 4.4 and 4.5). Mean values for C18:0 were 380, 289 and 306 mg/100 g muscle (sed = 38.1, $p<0.05$, Table 4.4), or 13.5, 12.0 and 11.8 % (sed = 0.37, $p<0.001$, Table 4.5) for Suffolk, Soay and Friesland, respectively. Similarly, the content of C12:0 was higher in Suffolk and Friesland than in the Soay lambs

with mean values of 6.5, 4.1 and 5.5 mg/100 g muscle (sed = 0.72, $p<0.01$, Table 4.4), or 0.23, 0.17 and 0.22 % (sed = 0.022, $p<0.05$, Table 4.5) for Suffolk, Soay and Friesland, respectively.

(d) Trans fatty acids

The concentrations of *trans* C18:1 was significantly higher in lambs offered all diets with added *n*-3 PUFA sources compared to lambs fed the Megalac diet when expressed either on a weight or percentage basis (Table 4.4 and 4.5). Mean values of *trans* C18:1 were 96.8, 148.9, 197.1 and 204.8 mg/100 g muscle (sed = 23.95, $p<0.001$) or 3.6, 6.0, 7.0 and 7.8 % (sed = 0.44, $p<0.001$) for lambs on the Megalac, linseed, fish oil and linfish diets, respectively. All the three breeds contained similar levels of *trans* C18:1, which was on average 162 g/100 g muscle or 6.1 % of the total fatty acids (Table 4.4 and 4.5).

4.4.1.2 Interaction effects

Soay lambs contained higher levels of C18:2*n*-6 and C22:6*n*-3 than the Suffolk or Friesland when offered the control (Megalac) diet but not when on the other dietary treatments (Table 4.4). Mean values for C18:2*n*-6 were 143.3, 182.8 and 127.7 and for C22:6*n*-3, 6.6, 11.6 and 7.3 mg/100 g muscle for the Suffolk, Soay and Friesland lambs, respectively (sed = 13.27 and 2.61, $p<0.05$ and $p<0.001$). These interactions were not significant when the data was expressed on a percentage basis but trends towards higher concentration of C18:2*n*-6 and C22:6*n*-3 in Soay than in Suffolk or Friesland lambs when on the control diet were observed. Mean values for C18:2*n*-6 were 5.3, 7.8 and 5.1 % (sed = 0.71, $p=0.06$), while mean values for C22:6*n*-3 were 0.25, 0.49 and 0.30 % (sed = 0.126, $p=0.08$) for Suffolk, Soay and Friesland, respectively (Table 4.5).

Table 4.4. *Effects of dietary fat and breed on the fatty acid composition of the semimembranosus muscle of sheep*

	mg/100 g muscle															
	Megalac			**Linseed**			**Fish oil**			**Linfish**				**Diet**	**Breed**	**[1]D x B**
Fatty acid	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**SED**	**sign**	**sign**	**sign**
C12:0	6.8	4.1	6.3	7.1	3.4	4.9	7.4	3.8	5.6	5.1	5.1	5.1	1.50	ns	**	ns
C14:0	76.0	61.3	74.0	70.3	54.7	57.0	92.4	64.4	76.1	68.1	81.6	61.1	16.13	ns	ns	ns
C16:0	675	586	656	553	465	484	769	563	687	596	672	516	115.4	ns	ns	ns
C16:1 n-7	49.8	51.3	54.6	45.2	39.8	44.0	83.4	55.7	72.7	48.8	67.0	45.5	11.87	**	ns	ns
C18:0	386	317	365	396	295	289	407	240	319	334	305	250	68.3	ns	*	ns
C18:1 *trans*	97.2	90.7	103.5	167.8	113.1	166.7	266.1	160.0	173.6	226.6	235.2	153.5	42.89	***	ns	ns
C18:1 *n*-9	953	745	930	843	648	734	812	541	763	736	744	639	141.8	ns	ns	ns
C18:1 *n*-7	31.9	28.0	35.6	27.8	23.8	28.9	68.6	42.8	52.8	38.5	41.2	33.0	7.36	***	ns	ns
C18:2 *n*-6	143.3	182.8	127.7	114.9	127.4	122.6	106.3	98.4	104.6	101.3	114.9	97.8	13.27	***	*	*
C18:3 *n*-3	42.8	51.4	35.2	90.0	87.2	79.8	42.5	40.7	33.5	63.0	57.2	47.8	9.34	***	*	ns
C20:3 *n*-6	3.8	5.2	4.3	2.6	3.9	3.6	6.6	6.5	6.4	3.1	4.9	3.7	0.67	***	**	ns
C20:4 *n*-6	35.1	44.2	38.5	25.6	35.7	33.7	22.7	30.8	26.6	24.5	32.5	29.1	3.95	***	***	ns
C20:4 *n*-3	1.4	2.0	3.8	6.4	3.3	4.1	47.1	39.6	40.5	12.6	19.3	13.3	4.86	***	ns	ns
C20:5 *n*-3	18.2	25.2	19.7	30.9	34.6	35.5	86.3	80.3	87.5	48.8	56.4	50.2	8.45	***	ns	ns
C22:4 *n*-6	1.7	1.5	2.2	0.8	1.4	1.0	1.1	1.7	1.2	1.0	1.4	1.7	0.39	*	ns	ns
C22:4 *n*-3	0.8	2.5	4.2	3.1	1.6	3.2	14.8	14.9	8.6	8.3	9.9	7.1	1.85	***	ns	**
C22:5 *n*-3	19.8	23.2	19.3	23.7	22.9	23.0	45.9	36.5	42.5	29.6	34.1	26.6	4.09	***	ns	ns
C22:6 *n*-3	6.6	11.6	7.3	11.1	12.4	15.4	34.2	21.7	24.7	20.4	19.8	17.3	2.61	***	ns	***
[2]WRFA	166	144	180	269	194	245	339	230	252	277	310	226	55.1	**	ns	ns
Total FA	2728	2406	2681	2700	2190	2391	3267	2298	2797	2657	2834	2241	459.5	ns	ns	ns

[1]D x B = Diet x Breed interaction; [2]WRFA = weight of remaining fatty acids

Table 4.5. *Effects of dietary fat and breed on the fatty acid composition of the semimembranosus muscle of sheep*

	% by weight of total fatty acids															
	Megalac			Linseed			Fish oil			Linfish				Diet	Breed	[1]D x B
Fatty acid	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	SED	sign	sign	sign
C12:0	0.24	0.17	0.23	0.26	0.15	0.20	0.23	0.17	0.20	0.18	0.19	0.23	0.045	ns	*	ns
C14:0	2.74	2.51	2.71	2.58	2.49	2.29	2.82	2.81	2.67	2.50	2.93	2.65	0.319	ns	ns	ns
C16:0	24.65	24.31	24.17	20.45	21.20	20.28	23.59	24.43	24.27	22.33	23.75	22.59	0.712	***	ns	ns
C16:1 *n*-7	1.80	2.10	2.04	1.67	1.82	1.83	2.50	2.40	2.59	1.82	2.34	2.07	0.149	***	**	ns
C18:0	14.17	13.26	13.08	14.66	13.41	12.00	12.39	10.46	11.03	12.52	10.77	10.99	0.772	***	***	ns
C18:1 *trans*	3.47	3.74	3.58	6.24	5.15	6.48	7.87	7.00	6.18	8.56	8.23	6.62	0.790	***	ns	ns
C18:1 *n*-9	35.05	30.81	34.78	31.07	29.59	31.01	24.31	23.42	26.97	27.64	26.28	28.70	1.449	***	***	ns
C18:1 *n*-7	1.20	1.16	1.48	1.05	1.09	1.27	2.07	1.87	1.99	1.47	1.44	1.55	0.184	***	ns	ns
C18:2 *n*-6	5.33	7.75	5.13	4.33	5.85	5.51	3.54	4.37	4.14	3.87	4.13	4.65	0.714	***	**	ns
C18:3 *n*-3	1.59	2.16	1.30	3.37	4.02	3.29	1.36	1.84	1.25	2.41	2.04	2.18	0.282	***	***	ns
C20:3 *n*-6	0.14	0.22	0.18	0.10	0.18	0.17	0.22	0.28	0.25	0.12	0.17	0.18	0.035	***	***	ns
C20:4 *n*-6	1.32	1.88	1.72	0.96	1.64	1.61	0.80	1.37	1.07	0.93	1.23	1.48	0.323	*	**	ns
C20:4 *n*-3	0.05	0.08	0.16	0.26	0.15	0.18	1.59	1.69	1.53	0.48	0.65	0.61	0.172	***	ns	ns
C20:5 *n*-3	0.69	1.07	0.79	1.17	1.60	1.52	2.99	3.54	3.30	1.88	1.97	2.37	0.363	***	*	ns
C22:4 *n*-6	0.06	0.07	0.11	0.03	0.06	0.06	0.03	0.07	0.06	0.03	0.05	0.09	0.023	ns	**	ns
C22:4 *n*-3	0.03	0.10	0.16	0.13	0.07	0.15	0.49	0.64	0.33	0.32	0.33	0.33	0.055	***	ns	***
C22:5 *n*-3	0.75	0.98	0.79	0.89	1.05	0.99	1.49	1.62	1.54	1.14	1.21	1.28	0.130	***	*	ns
C22:6 *n*-3	0.25	0.49	0.30	0.42	0.57	0.67	1.14	0.97	0.97	0.79	0.70	0.85	0.126	***	ns	ns
[2]RFA	5.99	5.91	6.65	9.93	8.86	9.81	10.07	9.91	8.98	10.42	10.81	9.75	0.760	***	ns	ns
Total FA	94.47	94.61	93.89	90.85	91.94	90.99	90.70	90.96	91.75	90.40	90.08	91.02	0.725	***	ns	ns

[1]D x B = Diet x Breed interaction; [2]RFA = remaining fatty acids

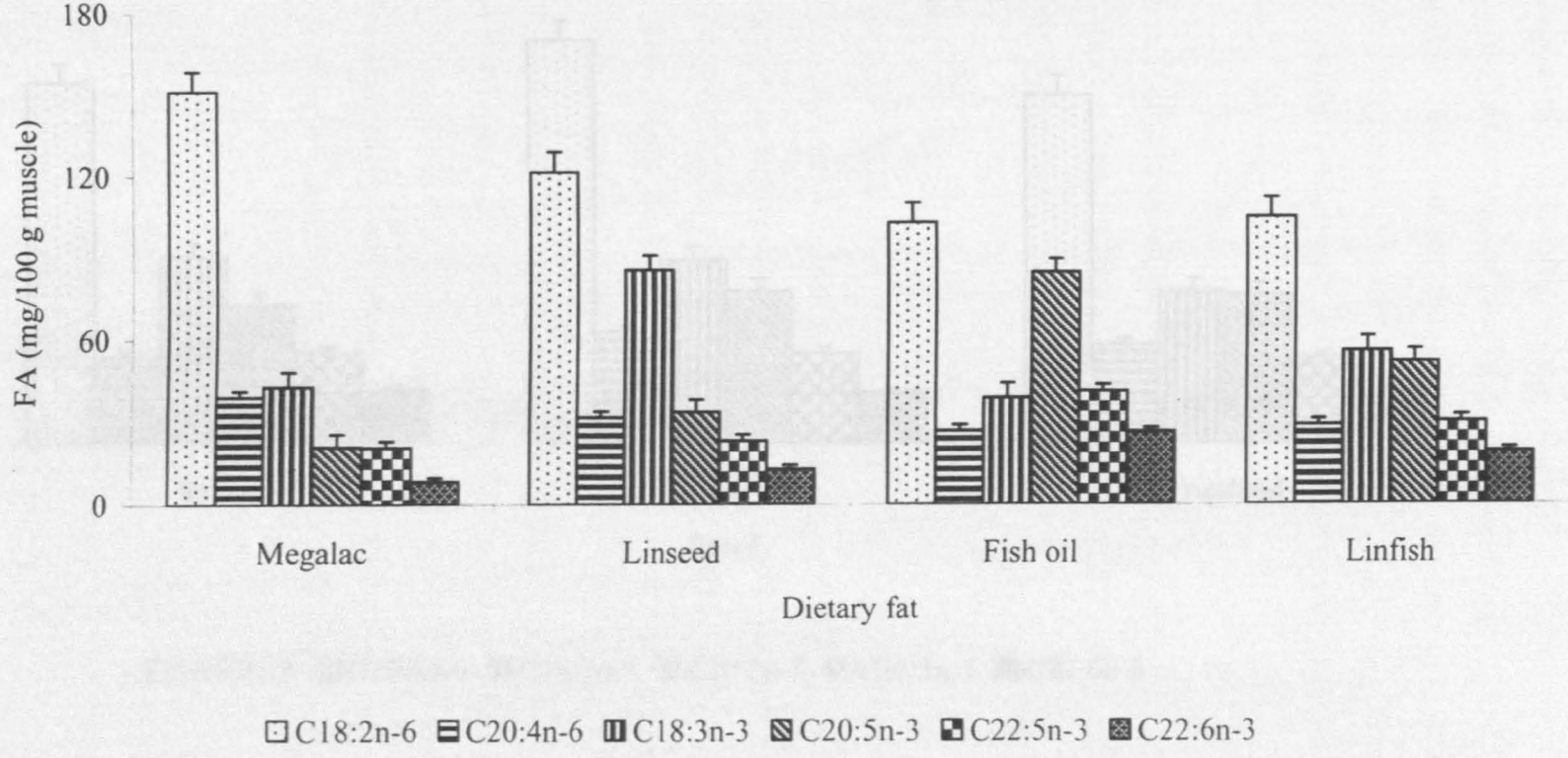


Figure 4.1 Fatty acid content of selected PUFA in the *semimembranosus* of lambs fed different dietary fats

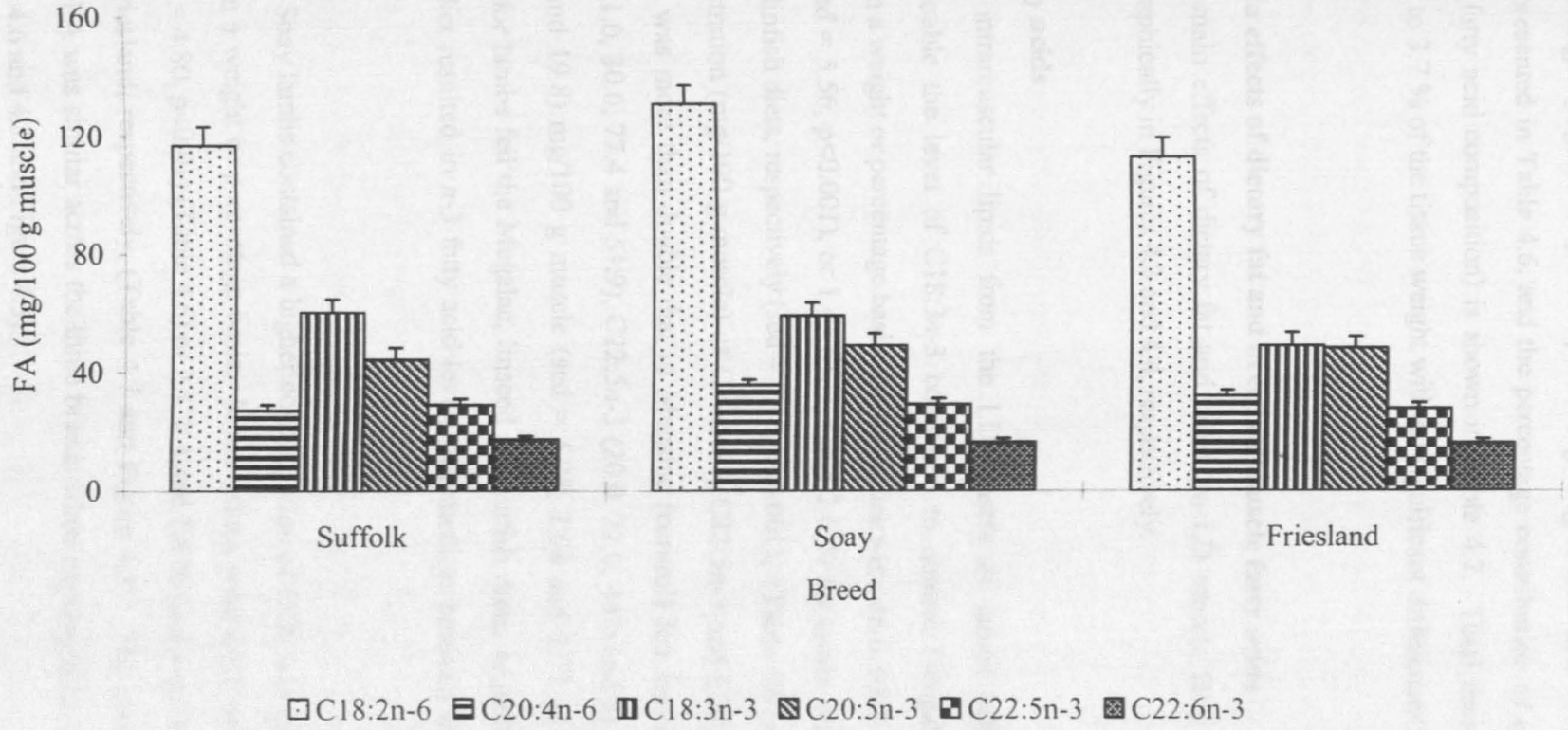


Figure 4.2 Breed effects on the content of selected PUFA in the *semimembranosus* of lamb

4.4.2 Fatty acid composition of *longissimus dorsi* muscle

The weight (mg) of fatty acids per 100 g *longissimus dorsi* (LD) muscle (fatty acid content) is presented in Table 4.6, and the percentage contribution of each fatty acid to total fatty acids (fatty acid composition) is shown in Table 4.7. Total muscle fatty acids ranged between 2.8 to 3.7 % of the tissue weight with no significant differences between treatments or breeds.

4.4.2.1 Main effects of dietary fat and breed on muscle fatty acids

The main effects of dietary fat and breed on LD muscle fatty acid composition are presented graphically in Figures 4.3 and 4.4, respectively.

(a) *n*-3 fatty acids

The intramuscular lipids from the LD muscle of lambs offered the linseed diet contained double the level of C18:3*n*-3 compared to control (Megalac) fed lambs, when expressed on a weight or percentage basis. Mean values were 46.6, 93.3, 45.2 and 62.8 mg/100 g muscle (sed = 5.56, $p<0.001$), or 1.4, 3.1, 1.4 and 2.0 % for lambs on the Megalac, linseed, fish oil and linfish diets, respectively (sed = 0.16, $p<0.001$), (Table 4.6 and 4.7 and Figure 4.3). The concentration (mg/100 g muscle) of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3, in lambs on the fish oil diet was more than double that in Megalac (control) fed lambs. Mean values were C20:5*n*-3 (21.0, 30.0, 77.4 and 51.9), C22:5*n*-3 (20.0, 22.0, 44.0 and 33.1) and C22:6*n*-3 (8.4, 11.7, 26.2 and 19.8) mg/100 g muscle (sed = 5.28, 2.68 and 1.77, $p<0.001$, Table 4.6 and Figure 4.3) for lambs fed the Megalac, linseed and linfish diets, respectively. Lambs offered the linfish diet resulted in *n*-3 fatty acid levels intermediate between the linseed and fish oil diets.

Suffolk and Soay lambs contained a higher concentration of C18:3*n*-3 than the Friesland when expressed on a weight or percentage basis. Mean values were 64.7, 66.0 and 56.1 mg/100 g muscle (sed = 4.80, $p<0.01$) (Table 4.6) or 2.1, 2.1 and 1.8 % (sed = 0.136, $p<0.01$) for Suffolk, Soay and Friesland, respectively, (Table 4.7 and Figure 4.3). The concentration of C20 and C22 *n*-3 PUFA was similar across the three breeds when expressed on a weight or percentage basis (Table 4.6 and 4.7 and Figure 4.3).

(b) *n*-6 fatty acids

Lambs on the Megalac diet contained the highest level of C18:2*n*-6, whilst lambs on the linseed diet contained higher levels than those fed either diet with fish oil when expressed on a weight or percentage basis. Mean values were 152.6, 117.4, 109.2 and 110.1 mg/100 g muscle (sed = 7.31, $p<0.001$, Table 4.6 and Figure 4.3), or 4.9, 4.0, 3.4 and 3.5 % (sed = 0.36, $p<0.01$, Table 4.7) for the Megalac, linseed, fish oil and linfish diets, respectively. Likewise, the concentration of C20:4*n*-6 was highest in lambs offered the Megalac diet when the data was expressed on a weight or percentage basis. Mean values were 33.5, 24.8, 25.0 and 26.0 mg/100 g muscle (sed = 2.16, $p<0.001$) or 1.1, 0.9, 0.8 and 0.9 % (sed = 0.16, $p<0.001$) for Megalac, linseed, fish oil and linfish diets, respectively, (Table 4.6 and 4.7 and Figure 4.3).

Soay lambs had higher intramuscular concentrations of all major *n*-6 PUFA than the Suffolk and Friesland, whilst Friesland lambs contained higher levels of C20:4*n*-6 than the Suffolk when expressed on a weight or percentage basis. Mean values for C18:2*n*-6 were 115.3, 135.7 and 116.3 mg/100 g muscle, (sed = 6.31, $p<0.001$, Table 4.6 and Figure 4.4), or 3.7, 4.2 and 3.8 % (sed = 0.31, $p<0.001$, Table 4.7), and mean values for C20:4*n*-6 were 22.5, 32.2 and 27.2 mg/100 g muscle, (sed = 1.86, $p<0.001$, Table 4.6 and Figure 4.4), or 0.7, 1.2 and 1.0 % (sed = 0.14, $p<0.001$, Table 4.7) for Suffolk, Soay and Friesland lambs, respectively.

(c) Monoenoic and saturated fatty acids

The concentration of the major monoenoic fatty acid, C18:1*n*-9 was highest in lambs offered the Megalac and linseed diets than in lambs fed the fish oil and linfish diets when the data was expressed on a weight basis, but when expressed as a percentage of the total fatty acids, lambs on the control diet contained the highest levels of C18:1*n*-9, whilst linseed fed lambs had higher levels than lambs offered both diets containing fish oil. Mean values for C18:1*n*-9 were, 1097, 938, 897 and 891 mg/100 g muscle (sed = 84.5, $p<0.01$, Table 4.6), and 34.1, 30.9, 25.9 and 27.4 % (sed = 0.93, $p<0.001$, Table 4.7), for Megalac, linseed, fish oil and linfish diets, respectively. By contrast lambs offered the fish oil diet had the highest concentration of C18:1*n*-7 and C16:1*n*-7, when expressed on a weight or percentage basis. Mean values for C18:1*n*-7 were 37.0, 31.8, 66.2 and 49.4 mg/100 g muscle (sed = 4.40, $p<0.001$, Table 4.6) or 1.2, 1.1, 2.0 and 1.5 % (sed = 0.12, $p<0.001$, Table 4.7), and mean values for C16:1*n*-7 were 61.6, 51.6, 82.4 and 66.7 mg/100 g muscle (sed = 6.59, $p<0.001$,

Table 4.6), or 1.9, 1.7, 2.4 and 2.0 % (sed = 0.09, $p<0.001$, Table 4.7) for lambs on the Megalac, linseed, fish oil and linfish diets, respectively.

The concentration of C18:1*n*-9 across the breeds was similar when expressed on a weight or percentage basis, with average values of 944, 948 and 977 mg/100 g muscle (Table 4.6), or 29.4, 28.6 and 30.9 % (Table 4.7), in Suffolk, Soay and Friesland lambs, respectively.

The concentration of C18:0 was highest in lambs offered the Megalac and linseed diets, where it constituted a significantly greater proportion of the total fatty acid, 14.5, 14.2, 11.9 and 12.1 % for lambs offered the Megalac, linseed, fish oil and linfish diets, respectively (sed = 0.51, $p<0.001$, Table 4.7), but the difference was not apparent when the data was expressed on a weight basis (Table 4.6). All three breeds contained a similar level of C18:0 when expressed on a weight basis (mean values were 449, 435 and 406 mg/100 g muscle) (Table 4.6), but on a percentage basis, Suffolk lambs had the highest C18:0 content with mean values of 14.1, 13.1 and 12.5 % (sed = 0.44, $p<0.001$, Table 4.7) for Suffolk, Soay and Friesland lambs, respectively.

When expressed on a weight basis the content of C16:0 was lower in lambs offered the linseed diet compared to lambs fed the fish oil diet (mean values for C16:0 were 828, 660, 869 and 787 mg/100 g muscle for lambs on the Megalac, linseed, fish oil and linfish diets, respectively, sed = 70.9, $p<0.01$, Table 4.6), and was also lower in lambs offered the linseed diet when expressed as a proportion of the total fatty acids, compared with lambs offered the Megalac, fish oil and linfish diets (mean values were 25.4, 21.8, 25.0 and 23.9 %, respectively for Megalac, linseed, fish oil and linfish diets, respectively, sed = 0.46, $p<0.001$, Table 4.7). Breed differences in C16:0 content were not apparent when the data was expressed on a weight basis, but when expressed as a percentage of the total fatty acids Suffolk lambs contained a lower proportion of C16:0 in their intramuscular lipids than the Soay lambs. Mean values were 23.4, 24.4 and 24.1 % (sed = 0.40, $p<0.01$, Table 4.7), for Suffolk, Soay and Friesland, respectively.

The content of C12:0 was higher in Suffolk and Friesland than in the Soay lambs with mean values of 5.8, 4.8 and 6.0 mg/100 g muscle (sed = 0.72, $p<0.01$), or 0.2, 0.1 and 0.2 % (sed = 0.02, $p<0.01$) for Suffolk, Soay and Friesland lambs, respectively (Table 4.6 and 4.7).

(d) Trans fatty acids

When expressed either on a weight or percentage basis (Table 4.6 and 4.7), the content of *trans* C18:1 was significantly higher in lambs offered all diets with added *n*-3 PUFA sources compared to lambs fed the Megalac diet, while lambs fed the linseed diet had lower levels than lambs offered the linfish diet. Mean values of *trans* C18:1 were 129.0, 200.8, 241.8 and 282.1 mg/100 g muscle (sed = 24.63, $p<0.001$) or 3.8, 6.6, 7.0 and 8.6 % (sed = 0.42, $p<0.001$) for lambs on the Megalac, linseed, fish oil and linfish diets, respectively. All the three breeds contained similar levels of *trans* C18:1, which was on average 213.0 g/100 g muscle or 6.5 % of the total fatty acids (Table 4.6 and 4.7).

4.4.2.2 Interaction effects

Soay lambs contained higher levels of C18:2*n*-6 and C18:3*n*-3 than the Friesland lambs when offered the control (Megalac) diet (Table 4.6 and 4.7). Mean values for C18:2*n*-6 were 128.1, 185.9 and 143.3 mg/100 g (sed = 13.09, $p<0.05$) and for C18:3*n*-3, 38.1, 58.1 and 43.7 mg/100 g muscle (sed = 9.96, $p<0.001$) for the Suffolk, Soay and Friesland lambs, respectively. These interactions were not significant when the data was expressed on a percentage basis but trends towards a higher concentration of C18:2*n*-6 and C18:3*n*-3 in Soay than in Friesland lambs when on the control diet were observed (Table 4.6 and 4.7).

Table 4.6. *Effect of dietary fat and breed on the fatty acid composition of the longissimus dorsi muscle of sheep*

	mg/100 g muscle															
	Megalac			**Linseed**			**Fish oil**			**Linfish**				**Diet**	**Breed**	**[1]D x B**
Fatty acid	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**Suffolk**	**Soay**	**Friesland**	**SED**	**sign**	**sign**	**sign**
C12:0	6.1	5.3	6.8	5.6	4.1	4.5	6.9	4.5	5.7	4.8	5.3	6.7	1.49	ns	Ns	ns
C14:0	82.0	87.7	84.6	73.9	79.1	67.0	98.6	85.6	95.3	71.7	98.7	83.3	16.95	ns	ns	ns
C16:0	797	890	795	657	700	623	891	781	941	710	899	751	126.9	**	ns	ns
C16:1 *n*-7	59.3	65.3	59.9	45.9	56.4	51.2	85.2	75.0	88.3	52.8	85.8	61.2	11.79	***	ns	*
C18:0	462	514	443	475	467	360	455	343	457	409	417	364	74.8	ns	ns	ns
C18:1 *trans*	124.7	144.4	118.2	200.4	181.6	220.7	296.6	222.5	214.3	274.4	329.6	242.6	44.10	***	ns	ns
C18:1 *n*-9	1075	1102	1114	969	977	867	898	765	1030	833	944	895	151.3	**	ns	ns
C18:1 *n*-7	38.7	32.5	40.0	29.5	31.1	34.7	73.5	56.8	69.5	47.4	54.3	46.5	7.88	***	ns	*
C18:2 *n*-6	143.3	185.9	128.1	109.3	127.3	115.2	111.0	107.3	110.4	98.5	121.3	110.1	13.09	***	***	*
C18:3 *n*-3	43.7	58.1	38.1	96.3	96.5	87.5	51.8	44.5	40.1	66.6	63.9	58.1	9.96	***	**	*
C20:3 *n*-6	3.8	4.3	3.8	2.3	2.8	3.3	5.9	6.6	5.6	2.7	4.8	3.4	0.70	*	***	*
C20:4 *n*-6	29.1	37.7	33.6	19.8	29.3	25.1	21.0	30.7	23.2	20.4	30.8	26.6	3.86	***	***	ns
C20:4 *n*-3	36.7	2.1	10.6	3.8	3.6	6.8	50.8	44.9	41.9	13.5	24.3	15.4	6.77	***	*	*
C20:5 *n*-3	23.3	21.2	18.3	26.5	29.6	34.0	83.7	74.4	75.4	44.9	60.0	50.5	9.46	***	ns	ns
C22:4 *n*-6	1.6	1.7	2.3	1.4	1.2	1.5	2.7	1.5	1.4	0.5	1.5	1.0	0.40	***	*	*
C22:5 *n*-3	20.5	20.8	18.4	21.5	21.3	23.3	49.8	39.3	43.8	30.0	39.0	30.2	4.79	***	ns	ns
C22:6 *n*-3	8.5	10.1	6.7	10.2	11.1	13.7	35.8	21.5	22.7	19.9	20.7	18.7	3.16	***	*	*
[2]WRFA	199.0	198.7	197.4	329.7	277.9	301.6	417.7	355.8	338.7	313.4	410.6	337.3	56.80	***	ns	ns
Total FA	3148	3416	3135	3095	3123	2862	3659	3093	3626	3030	3641	3125	476.1	ns	ns	ns

[1]D x B = Diet x Breed interaction; [2]WRFA = weight of remaining fatty acids

Table 4.7. *Effect of dietary fat and breed on the fatty acid composition of the longissimus dorsi muscle of sheep*

% by weight of total fatty acids

	Megalac			Linseed			Fish oil			Linfish				Diet	Breed	[1]D x B
Fatty acid	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	SED	sign	sign	sign
C12:0	0.19	0.15	0.21	0.18	0.13	0.15	0.20	0.14	0.16	0.15	0.15	0.22	0.042	ns	**	ns
C14:0	2.53	2.52	2.61	2.38	2.52	2.30	2.68	2.70	2.60	2.31	2.74	2.64	0.311	ns	ns	ns
C16:0	25.08	25.97	25.12	21.14	22.30	21.89	24.29	24.88	25.73	23.17	24.56	23.78	0.819	***	**	ns
C16:1 *n*-7	1.85	1.89	1.94	1.47	1.81	1.82	2.32	2.38	2.45	1.74	2.36	1.97	0.161	***	***	ns
C18:0	14.65	15.07	13.81	15.55	14.85	12.37	12.47	11.18	12.12	13.41	11.37	11.67	0.912	***	***	ns
C18:1 *trans*	3.82	4.23	3.44	6.41	5.93	7.50	8.10	7.06	6.01	9.03	9.10	7.64	0.873	***	ns	ns
C18:1 *n*-9	34.09	32.10	36.09	31.28	30.92	30.38	24.36	25.17	28.03	27.31	26.06	28.84	1.671	***	***	*
C18:1 *n*-7	1.30	0.96	1.40	0.94	1.03	1.26	2.03	1.82	1.98	1.56	1.49	1.49	0.207	***	*	*
C18:2 *n*-6	4.75	5.60	4.25	3.57	4.22	4.14	3.13	3.66	3.29	3.35	3.43	3.66	0.650	***	ns	*
C18:3 *n*-3	1.44	1.73	1.16	3.13	3.16	2.99	1.43	1.54	1.15	2.28	1.82	1.86	0.283	***	*	ns
C20:3 *n*-6	0.12	0.13	0.13	0.08	0.09	0.12	0.17	0.21	0.17	0.09	0.13	0.11	0.031	***	***	*
C20:4 *n*-6	0.99	1.14	1.24	0.66	0.97	0.94	0.59	1.08	0.72	0.68	0.90	0.96	0.284	***	***	ns
C20:4 *n*-3	1.26	0.06	0.30	0.11	0.13	0.26	1.43	1.38	1.22	0.46	0.61	0.49	0.203	***	***	ns
C20:5 *n*-3	0.81	0.65	0.57	0.85	1.00	1.23	2.36	2.38	2.22	1.54	1.63	1.66	0.347	***	ns	ns
C22:4 *n*-6	0.06	0.05	0.10	0.06	0.04	0.08	0.10	0.05	0.05	0.01	0.04	0.04	0.023	**	*	*
C22:5 *n*-3	0.70	0.63	0.62	0.69	0.70	0.84	1.37	1.27	1.24	1.02	1.05	1.00	0.134	***	ns	ns
C22:6 *n*-3	0.30	0.31	0.22	0.33	0.37	0.50	0.98	0.71	0.69	0.68	0.57	0.63	0.121	***	ns	ns
[2]RFA	6.29	5.78	6.12	10.60	8.98	10.43	11.38	11.32	9.49	10.59	11.10	10.54	0.8210.7	***	ns	ns
Total FA	94.19	94.67	94.35	90.26	91.75	90.45	89.45	89.56	91.21	90.30	89.74	90.29	71	***	ns	ns

[1]D x B = Diet x Breed interaction; [2]RFA = remaining fatty acids

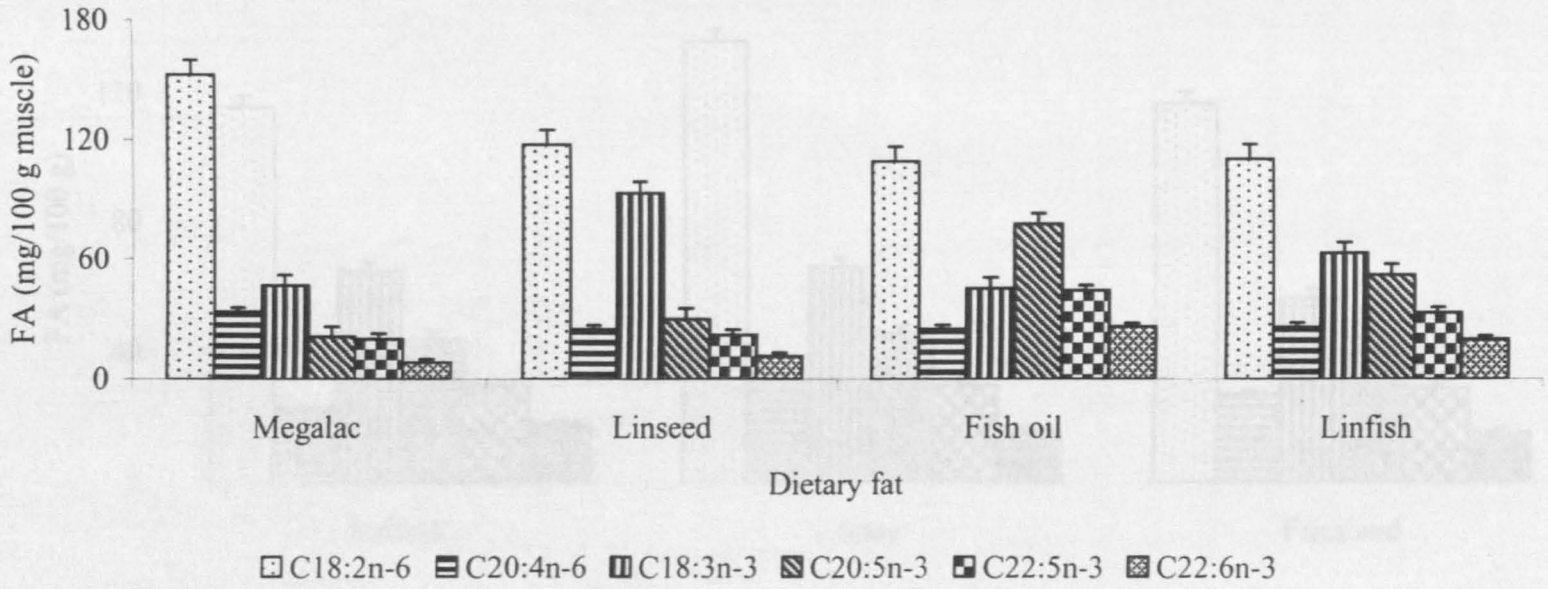


Figure 4.3 Fatty acid content of selected PUFA in the *longissimus dorsi* of lambs fed different dietary fats

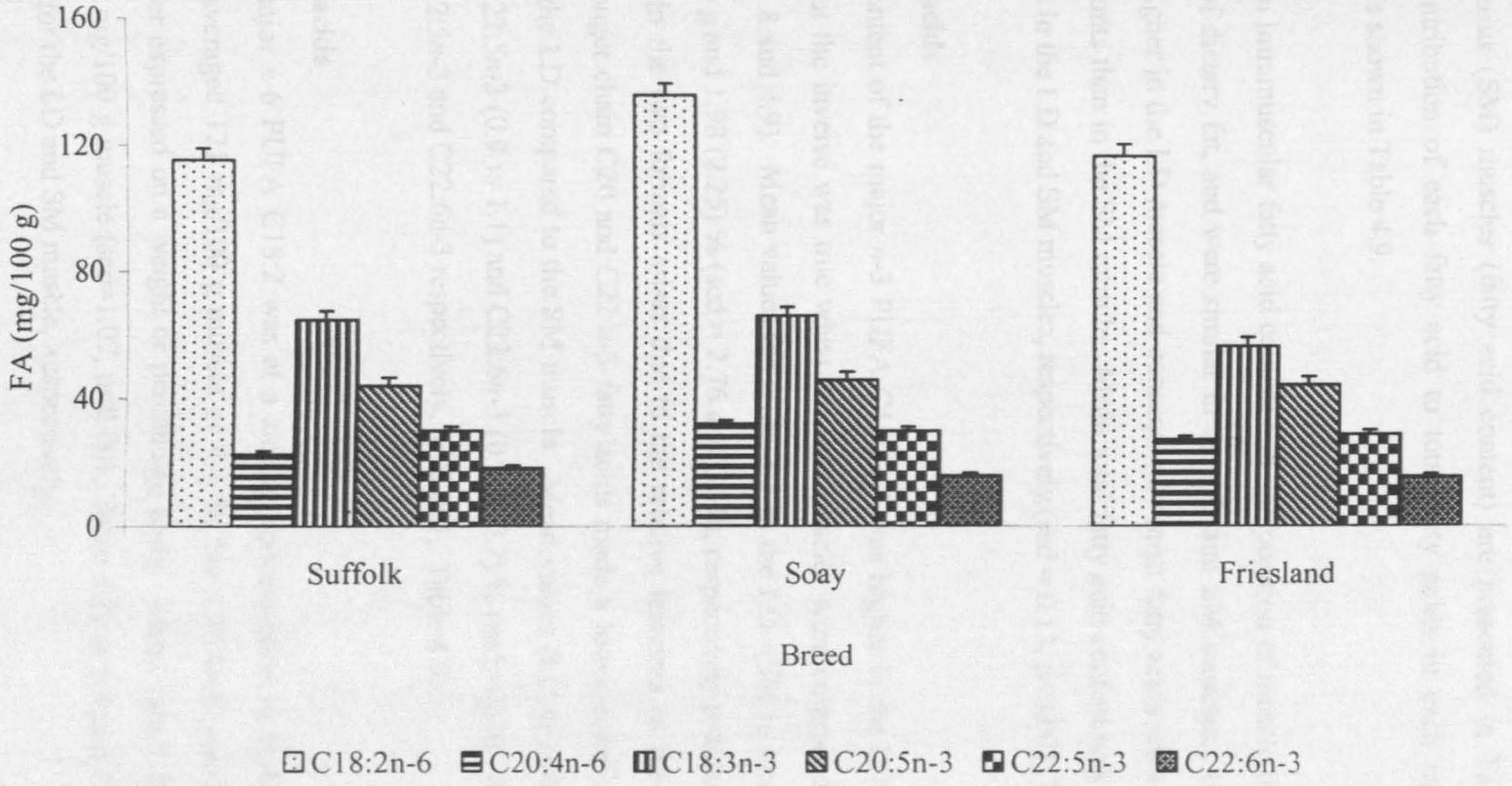


Figure 4.4 Breed effects on content of selected PUFA in the *longissimus dorsi* of lamb

4.4.3 Differences in fatty acid composition between muscles

The weight (mg) of fatty acids per 100 g of *longissimus dorsi* (LD) and *semimembranosus* (SM) muscles (fatty acid content) are presented in Table 4.8, and the percentage contribution of each fatty acid to total fatty acids in each muscle (fatty acid composition) is shown in Table 4.9.

The changes in intramuscular fatty acid content and composition of muscle lipids reflected the composition of dietary fat, and were similar in the LD and SM muscles. However, total fat content was higher in the LD muscle and consequently most fatty acids were therefore present in higher amounts than in the SM muscle. Mean total fatty acid content was 3.2 and 2.6 % of muscle weight in the LD and SM muscles, respectively (sed = 0.13, $p<0.001$, Table 4.8).

(a) *n*-3 fatty acids

The content of the major *n*-3 PUFA C18:3*n*-3 was higher in the LD compared to the SM muscle but the inverse was true when the two muscles were compared on a percentage basis (Table 4.8 and 4.9). Mean values for C18:3*n*-3 in the LD (SM in brackets) were, 62.2 (56.1) mg/100 g and 1.98 (2.25) % (sed = 2.76 and 0.078, respectively $p<0.001$). The apparent contradiction in the two formats arises due to the relative leanness of the SM muscle. In contrast the longer chain C20 and C22 *n*-3 fatty acids made a lower contribution to the total fatty acids in the LD compared to the SM muscle. Mean values (LD *vs* SM) were: C20:5*n*-3 (1.4 *vs* 1.9), C22:5*n*-3 (0.9 *vs* 1.1) and C22:6*n*-3 (0.5 *vs* 0.7) % (sed = 0.10, 0.04 and 0.03, for C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 respectively, $p<0.001$, Table 4.9).

(b) *n*-6 fatty acids

The major *n*-6 PUFA C18:2 was at a similar concentration in both the LD and SM muscles and averaged 121 mg/100 g muscle or 4.4 %, but C20:4*n*-6 was higher in the SM muscle whether expressed on a weight or percentage basis. Mean values for C20:4*n*-6 were 27.4 and 31.7 mg/100 g muscle (sed=1.07, $p<0.001$, Table 4.8) or 0.9 and 1.3 % (sed = 0.08, $p<0.001$, 4.9) for the LD and SM muscle, respectively.

(c) Monoenoic and saturated fatty acids

The major fatty acid C18:1*n*-9, predominated in the LD over the SM muscle, when the fatty acids were expressed as a proportion of muscle weight but the difference was not apparent

when expressed on a percentage basis (Table 4.9). Mean values (mg/100 g muscle) were 956 and 757 for the LD and SM muscle, respectively (sed = 41.9, $p<0.001$, Table 4.8). Similarly the contents of C18:1*n*-7 and C16:1*n*-7 were higher in the LD than SM muscle when expressed on a weight basis (but not when on a percentage basis) with mean values (mg/100 g muscle) of 45.8 *vs* 37.3 for C18:1*n*-7 (sed = 2.18, $p<0.001$, Table 4.8) and 65.2 *vs* 54.4 for C16:1*n*-7 (sed = 3.27, $p<0.001$, Table 4.8), for the LD *vs* SM muscles, respectively.

The LD muscle also contained a higher concentration of the major saturated fatty acids C16:0 and C18:0 when expressed on a weight or percentage basis. Mean values for C16:0 were 785 *vs* 600 mg/100 g muscle (sed = 35.2, $p<0.001$, Table 4.8) or 24.0 *vs* 23 % (sed = 0.23, $p<0.001$, Table 4.9), and mean values for C18:0 were 430 *vs* 324 mg/100 g muscle (sed = 20.7, $p<0.001$, Table 4.8) or 13.22 *vs* 12.39 % (sed = 0.25, $p<0.001$, Table 4.9), for the LD *vs* SM muscle, respectively.

(d) Trans fatty acids

The level of *trans* C18:1 was higher in the LD than in the SM muscle when expressed on a weight basis but the difference was not apparent when expressed as a proportion of the total fatty acids. Mean values were 213 *vs* 161.4 mg/100 g muscle (sed = 12.22, $p<0.001$, Table 4.8), for the LD and SM muscle, respectively.

Table 4.8. *Effect of dietary fat on the fatty acid composition of the longissimus and the semimembranosus muscle of sheep*

mg per 100 g muscle

	Megalac		Linseed		Fish oil		Linfish			Fat	Muscle	Fat x Muscle
Fatty acid	[5]**LD**	[6]**SM**	**LD**	**SM**	**LD**	**SM**	**LD**	**SM**	**SED**	**sign**	**sign**	**sign**
C12:0 lauric	6.1	5.7	4.7	5.1	5.6	5.5	5.6	5.1	0.833	ns	ns	ns
C14:0 myristic	84.8	70.3	73.4	60.6	92.7	77.0	84.6	70.2	9.47	ns	**	ns
C16:0 palmitic	828	639	660	500	869	669	787	594	70.9	**	***	ns
C16:1 *n*-7 palmitoleic	61.6	51.9	51.3	43.0	82.4	69.9	66.7	53.8	6.59	***	***	ns
C18:0 stearic	473	355	434	326	417	320	396	296	41.8	ns	***	ns
C18:1 *trans*	129.0	96.8	200.8	148.9	241.8	197.1	282.1	204.8	24.63	***	***	ns
C18:1 *n*-9 oleic	1097	875	938	741	897	703	891	705	84.5	**	***	ns
C18:1 *n*-7 vaccenic	37.0	31.8	31.8	26.8	66.2	54.1	49.4	37.5	4.40	***	***	ns
C18:2 *n*-6 linoleic	152.6	151.3	117.4	121.7	109.2	102.8	110.1	104.7	7.31	***	ns	ns
C18:3 *n*-3 linolenic	46.6	43.1	93.3	85.6	45.2	38.9	62.8	55.9	5.56	***	*	ns
C20:3 *n*-6	3.8	4.4	2.8	3.4	6.0	6.4	3.6	3.9	0.38	***	*	ns
C20:4 *n*-6 arachidonic	33.5	39.3	24.8	31.7	25.0	26.8	26.0	28.8	2.16	***	***	ns
C20:4 *n*-3	16.4	2.4	4.7	4.6	45.8	42.1	17.7	15.1	3.78	***	**	*
C20:5 *n*-3 EPA[1]	20.9	21.0	30.0	33.7	77.4	84.5	51.9	51.9	5.28	***	ns	ns
C22:4 *n*-6	1.9	1.8	1.4	1.3	1.8	1.9	1.0	1.4	0.22	***	ns	ns
C22:5 *n*-3 DPA[2]	19.9	20.8	22.0	23.2	44.0	41.4	33.1	30.1	2.68	***	ns	ns
C22:6 *n*-3 DHA[3]	8.4	8.5	11.7	13.0	26.2	26.5	19.8	19.2	1.77	***	ns	ns
[7]WRFA	198.3	163.1	303.0	236.9	368.2	283.7	353.7	278.1	31.73	***	***	ns
Total fatty acids	3233	2602	3027	2424	3446	2769	3266	2574	265.9	ns	***	ns

[1]EPA=Eicosapentaenoic acid; [2]DPA=Docosapentaenoic acid; [3]DHA=Docosahexaenoic acid[5]LD= *Longissimus dorsi*; muscle [6]SM= *Semimembranosus* muscle; [7]WRFA weight of remaining fatty acids

Table 4.9 *Effect of dietary fat on fatty acid composition of the longissimus and the semimembranosus muscle of sheep*
% by weight of total fatty acids

	Megalac		Linseed		Fish oil		Linfish			Fat	Muscle	Fat x Muscle
Fatty acid	[5]LD	[6]SM	LD	SM	LD	SM	LD	SM	SED	sign	sign	sign
C12:0 lauric	0.18	0.21	0.15	0.20	0.16	0.20	0.17	0.20	0.237	Ns	**	Ns
C14:0 myristic	2.56	2.65	2.40	2.45	2.65	2.76	2.57	2.69	0.174	ns	ns	ns
C16:0 palmitic	25.40	24.38	21.78	20.65	24.98	24.11	23.85	22.89	0.457	***	***	ns
C16:1 *n*-7 palmitoleic	1.90	1.98	1.70	1.78	2.38	2.49	2.02	2.08	0.090	***	ns	ns
C18:0 stearic	14.50	13.49	14.24	13.34	11.93	11.28	12.14	11.41	0.509	***	***	ns
C18:1 *trans*	3.83	3.59	6.61	5.95	7.01	6.99	8.59	7.80	0.488	***	ns	ns
C18:1 *n*-9 oleic	34.09	33.54	30.86	30.55	25.93	24.96	27.41	27.53	0.933	***	ns	ns
C18:1 *n*-7 vaccenic	1.22	1.28	1.07	1.14	1.94	1.97	1.51	1.49	0.116	***	ns	ns
C18:2 *n*-6 linoleic	4.87	6.08	3.98	5.24	3.36	4.02	3.48	4.23	0.363	***	***	ns
C18:3 *n*-3 linolenic	1.44	1.68	3.09	3.56	1.37	1.49	1.99	2.21	0.158	***	***	ns
C20:3 *n*-6	0.13	0.18	0.10	0.15	0.18	0.25	0.11	0.16	0.017	***	***	ns
C20:4 *n*-6 arachidonic	1.13	1.65	0.86	1.41	0.80	1.08	0.85	1.22	0.159	***	***	ns
C20:4 *n*-3	0.54	0.10	0.16	0.20	1.35	1.60	0.52	0.58	0.113	***	ns	***
C20:5 *n*-3 EPA[1]	0.68	0.85	1.03	1.43	2.32	3.28	1.61	2.08	0.194	***	***	*
C22:4 *n*-6	0.07	0.08	0.06	0.06	0.07	0.08	0.03	0.06	0.013	**	ns	ns
C22:5 *n*-3 DPA[2]	0.65	0.84	0.75	0.98	1.29	1.55	1.03	1.21	0.075	***	***	ns
C22:6 *n*-3 DHA[3]	0.27	0.35	0.40	0.55	0.78	1.02	0.63	0.78	0.068	***	***	ns
Remaining fatty acids	6.06	6.20	10.00	9.59	10.71	10.12	10.74	10.62	0.458	***	ns	ns
Total fatty acids	94.41	94.31	90.82	91.20	90.10	90.71	90.11	90.23	0.430	***	ns	ns

[1]EPA=Eicosapentaenoic acid; [2]DPA=Docosapentaenoic acid; [3]DHA=Docosahexaenoic acid;[5]LD= *Longissimus dorsi*; muscle [6]SM= *Semimembranosus* muscle

4.6 DISCUSSION

4.6.1 Animal performance and carcass characteristics

The lower food intake in lambs offered diets containing fish oil in the current work agree with trends reported in the literature (Kowalczyk *et al.* 1977; Knight *et al.* 1978; Ikwuegbu and Sutton, 1982) and with the negative effects on microbial growth observed on rumen metabolism reported in Chapter 3. This finding has been attributed to a reduction in fibre digestion, which affects the retention time of food in the rumen and consequently affects voluntary food intake. As previously discussed (Chapter 3, section 3.4.6) the negative effects of PUFA on rumen metabolism can be attributed to their toxic effects in the rumen which limit microbial efficiency and are probably the main factors limiting the amount and composition of fat which can be added to ruminant diets.

Despite these deleterious effects of fish oil on dry matter intake (DMI), feed conversion ratio was similar in lambs fed any of the four dietary treatments. Nicholson *et al.* (1992) reported a similar response with fishmeal supplementation depressing DMI but improving feed conversion efficiency by increasing the efficiency of utilization of absorbed nutrients. Doreau and Chilliard (1997) have recently reported significant increases in dry matter, organic matter and fibre digestibility after feeding cows diets supplemented with 400 ml of fish oil compared to unsupplemented controls. Such positive effects of fish oil are thought to be mediated by specific fatty acids which are produced during the biohydrogenation of fish oil in the rumen rather than the overall increased nutrient digestibility. One such fatty acid is conjugated linoleic acid (CLA), an intermediate in the biohydrogenation of dietary linoleic acid to stearic acid in the rumen. Feeding weaner rats on diets supplemented with CLA resulted in greater body weight gain, improved feed efficiency and more lean body mass per unit of consumed feed than unsupplemented controls (Chin *et al.* 1994). The CLA effects are exerted through a reduction of catabolic effects of immune stimulation without adversely affecting immune function (Cook *et al.* 1993). Immune stimulation induces catabolism of skeletal muscle, which in turn partitions energy away from other biological processes, including growth.

In human nutrition CLA has several unique structural and functional properties resulting in chemical and physiological effects that are different from those of all-*cis* nonoconjugated PUFA. Because CLA is readily incorporated into cell membrane phospholipids it may inhibit

carcinogenesis by modulating several cellular events that are mediated in part by the plasma membrane lipids (Belury, 1995). In the plasma membrane, the replacement of other PUFA with CLA may ultimately affect events such as oxidative stress, eicosanoid synthesis and signal transduction (Merrill and Schroeder, 1993). In bacteria, it is thought that the conversion of C18:2*n*-6 to CLA via biohydrogenation decreases reducing capabilities in the cell. Although this function of CLA has not been clearly demonstrated, compared with other antioxidants CLA has been to be more potent that α-tocopherol and as effective as butylated hydroxytolulene (BHT) in inhibiting iron-thiocyanate induced peroxide formation ((Ha *et al.* 1990). In addition CLA was shown to be as effective as vitamin E and butylated hydroxyanisole in inhibiting the formation of thiobarbituric acid reactive substances (TBARS), a biomarker often used to assess oxidation in biological systems (Ip *et al.* 1991). The effects of dietary CLA on eicosanoid synthesis are not yet well understood.

Lambs offered diets containing fish oil had a significantly higher fat score than those offered either the control or linseed diets. This may have resulted from an increased maturity at slaughter due principally to the lower live weight gain in lambs offered the fish oil diet, which would have resulted in a greater partitioning of energy towards fat deposition rather than protein accretion (McClelland *et al.* 1976). According to Butler-Hogg and Johnson, (1986) both growth rate and age affect carcass fatness with older lambs of equal weight, and faster growing lambs of equal weight and age containing higher levels of carcass fat. Lambs with a high level of growth (lambs offered control, linseed and linfish diets) in the present study attained half the mature live weight faster in comparison with their counterparts with lower growth rates (lambs fed on fish oil diet). Mean number of days on the respective dietary treatments was 65 for lambs on the control, linseed and linfish diets compared to 85 for lambs on the fish oil diets. Carcass fatness is reported to increase with fishmeal supplementation (Comerford *et al.* 1992) but was attributed more to a higher ME intake rather than dietary ME content by Solomon *et al.* (1992). However, Rule *et al.* (1994) found that high fat diets did not necessarily increase carcass fatness.

The inclusion of fish oil was shown in the previous metabolism experiment to alter the acetate to propionate ratios (Chapter 3). Although acetate is the normal substrate for fatty acid synthesis yielding even chain length saturated acids, propionate can replace acetate to a limited extent either as a primer, in which case an odd chain length fatty acid is produced (not

measured in this study) or as methyl malonyl coenzyme A (Garton *et al.* 1972). Factors controlling this process are not fully understood but some breeds have higher levels of odd chain and branched chain fatty acids than others and rams have more than castrates or females (Enser, 1991). The increases in propionate increase gluconeogenesis which favours lipogenesis and consequently increased fat deposition, especially subcutaneous fat which has the highest growth coefficient, 1.23 compared to 0.74 for intermuscular fat in growing lambs (Jones, 1982). Several other studies have reported that lambs on high-concentrate diets had fatter carcasses than lambs on high-roughage diets and this effect was attributed to increased propionate in the rumen (Solomon *et al.* 1986)

Most if not all the significant breed effects on carcass characteristics were due to the small Soay breed, which was typified by low subcutaneous fat, low intermuscular fat and a higher proportion of lean (muscle). Similar findings have been reported by McClelland *et al.* (1976) and Thonney *et al.* (1987), where Soay lambs were found to have a higher proportion of commercially valued cuts in the carcass but lower proportions of internal fat. The Soay lambs were thus expressing a real breed difference in body composition in that they do not deposit large amounts of body fat. This could be a feature of the natural environment under which the animal exists in the wild, which has selected lean animals due to the limited food resources.

Carcass weights of Suffolk and Friesland lambs were within the average range (16-19.9 kg) of lambs at slaughter in the UK. Apart from the Soay breed, all carcasses were graded R3L the most common classification accounting for 26 % of the classified lamb carcasses (Meat and Livestock Commission, 1993). However different breeds of sheep have been shown to differ in their fat partition. Wood *et al.* (1980) demonstrated that meat sire breeds (Suffolk, Hampshire) had less internal body fat than ewe-type breeds (Clun, Colbred). The latter breeds thus had a 'poor' conformation compared to the meat type breeds, as observed with the Friesland in the current study. Although the Suffolk and Friesland breeds have been subjected in the past to different kinds of pressure from artificial selection, the amounts of body fat was similar and averaged 10 %. This finding supports the earlier hypothesis of McClelland *et al.* (1972) that at a stipulated degree of maturity variations in body composition between breeds were minimal.

4.6.2 Muscle fatty acids

4.6.2.1 *Semimembranosus* and *longissimus* muscle fatty acids

Percentage figures for fatty acids can be misleading when muscles differ in total fatty acid content. This is because at low levels of fat the contribution made by phospholipids is proportionally greater and these are more unsaturated than the neutral lipids which increase in proportion to total lipid increases (Marmar *et al.* 1984). Hence, the differences in fatty acid composition between muscles was relatively small and reflected differences in phospholipid concentration, which is greater in red oxidative muscle fibres (*semimembranosus* muscle) than in glycolytic muscle or whiter muscles (*longissimus dorsi* muscle) (Tukii and Campbell, 1967). The leaner *semimembranosus* muscle contained higher percentages of all the PUFA while the *longissimus* muscle contained higher percentages of major saturated and monounsaturated fatty acids. The absence of differences in fatty acid composition between the muscles was similar to that reported in lambs for total intramuscular fatty acids in carcasses of widely different fat levels (Solomon *et al.* 1991) and also in grass fed lambs in the study of Enser *et al.* (1998). Therefore, the results from the two muscles can be discussed together.

4.6.2.2 Effects of diet and breed on muscle fatty acids

Despite extensive biohydrogenation of C18:3*n*-3 in the previous study (Chapter 3), the duodenal flow of C18:3*n*-3 in lambs fed the linseed diet was twice that recorded on the Megalac (control) diet. This difference was also reflected in the intramuscular fatty acids, where lambs offered the linseed diet had more than double the concentration C18:3*n*-3 in lambs on the Megalac diet. Similarly, the levels of C20:5*n*-3 and C22:6*n*-3 were approximately four times greater in lambs offered the fish oil diet compared to lambs offered the Megalac diet. This suggests an efficient conservation of dietary *n*-3 fatty acids as well as efficient incorporation into muscle lipids, a result in accordance with that demonstrated by Reid and Husbands, (1985). Ruminants have only 0.3 to 0.5 % of their dietary energy entering the duodenum as essential fatty acids (EFA) due to rumen biohydrogenation (Leat and Harrison, 1972), yet show no signs of EFA deficiency. Previously it was demonstrated that ruminants conserve EFA more efficiently than non-ruminants which require at least 1-2 % of their dietary energy as EFA to avoid signs of deficiency (Holman, 1968).

The observed increases in muscle PUFA in this study are higher than reported when diets supplemented with oilseeds were offered to steers (Rule and Beitz, 1986; St. John *et al.* 1987; Ekeren *et al.* 1992) and sheep (Solomon *et al.* 1991; Lough *et al.* 1992). These discrepancies maybe due to variability caused by duration of feeding, initial and finish slaughter live weights because most reported studies have been conducted in the US where animals are fatter than in Europe. For example, the slaughter live weight was 70 kg in a study by Solomon *et al.* (1992) using Suffolk x Hampshire lambs compared to 35-42 kg which is the average slaughter weight in the UK, and 42 kg in the current study for the Suffolk crosses. The level of fatness can itself affect the fatty acid composition of total lipid because the infiltrated fat (neutral lipid fraction), which increases with increasing fatness, is more saturated than the more constant intramuscular phospholipid fraction (Marmer *et al.* 1984). Neutral lipids (triacylglycerides) are present in the intramuscular adipose tissue, marbling fat and microscopic lipid droplets between the muscle fibres. The proportion of neutral lipids can vary from less than 1 to over 30 % of tissue weight, although for UK meat 10 % is rarely exceeded (Enser and Wood, 1997). In contrast the structural lipids or phospholipid are relatively constant and comprise 0.5 to 1.0 % of the muscle weight.

Compared to the control fat, lambs on the whole linseed diet had higher levels of C18:3*n*-3 and its desaturation/elongation products, particularly C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3. The effects of whole linseed on fatty acid composition were similar to those in beef as reported by Scollan *et al.* (1997) using similar diets or by Enser *et al.* (1998) in grazed lambs, but the relative proportions of *n*-3 PUFA were higher in this study. The concentration of C20:5*n*-3 and C22:5*n*-3 however, significantly increased without proportional increases in C22:6*n*-3 which suggests a close regulation of the conversion of C20:5*n*-3 to C22:6*n*-3. Mandell *et al.* (1997) also observed that although fish meal increased the proportion of C22:6*n*-3 in the *longissimus* muscles the length and duration of feeding was not significant. In rats the maximum concentration of C22:6*n*-3 from the diet occurred when the ratio of C18:2*n*-6 to C18:3*n*-3 in the diet was about 2.7 (Ratnayake *et al.* 1992).

Levels of C18:2*n*-6 and C20:4*n*-6, its elongation and desaturation metabolite, decreased when animals were offered diets containing C20:5*n*-3 (fish oil and linfish diets) in muscle lipids. In contrast the linseed diet had only a marginal influence on muscle C20:4*n*-6 concentration, which was not significant. The *n*-6 and *n*-3 precursor fatty acids, C18:2*n*-6 and C18:3*n*-3

respectively, compete for Δ6 desaturase and consequently incorporation into tissue lipids (Brenner, 1977). Therefore, the lower levels of C20:4*n*-6 in lambs fed diets containing fish oil reflects the inhibition of C18:2*n*-6 desaturation by preformed long chain *n*-3 fatty acids.

The main sequence of reactions in the desaturation and elongation of *n*-3 and *n*-6 series fatty acids begins with Δ6 desaturation followed alternately by elongation, Δ5 desaturation and another Δ4 desaturation (Brenner, 1989). Studies both *in vivo* and *in vitro* have demonstrated that competition for Δ6 desaturases is in the order of *n*-3> *n*-6> *n*-9 series (Sprecher, 1977). In the current study, this would account for the increased synthesis of *n*-3 PUFA from the relatively low levels of C18:3*n*-3 escaping rumen biohydrogenation. Desaturation is however slower than elongation because concentrations of C20:5 and C22:6 *n*-3 fatty acids are always lower than C18:3*n*-3 concentrations (Rule *et al.* 1994). This implies that addition of a double bond rather than elongation is the rate limiting step in the conversion of C18:3*n*-3 to longer chain more unsaturated fatty acids. As a result feeding preformed longer chain PUFA in fish oil increased intramuscular concentrations of longer chain *n*-3 PUFA more than feeding C18:3*n*-3 in the linseed diet.

The higher concentration of C18:2*n*-6 and other *n*-6 fatty acids in the intramuscular lipids of lambs offered the control diet reflected the higher levels of dietary C18:2*n*-6 from soybean meal. Similar observations were reported while feeding diets containing whole rapeseed, soybean meal and other cereal based diets (St. John *et al.* 1987; Solomon *et al.* 1991; Lough *et al.* 1992). Enser *et al.* (1998) observed that while concentrate fed ruminants contained high levels of intramuscular *n*-6 fatty acids, grazing ruminants were high in *n*-3 fatty acids, reflecting differences in dietary fatty acids. In the absence of adequate amounts of *n*-3 fatty acids for absorption, available *n*-6 fatty acids are absorbed and deposited in the phospholipid fraction of intramuscular lipids. On the contrary, feeding protected fish oil supplements to sheep in the work of Ashes *et al.* (1992b) did not reduce the concentration of C20:4*n*-6 in the phospholipid fraction. The authors attributed this finding to lack of Δ6 desaturase inhibition by the elevated C20:5*n*-3 levels. However, although increased levels of C18:2*n*-6 favour its conversion to longer chain *n*-6 fatty acid particularly C20:4*n*-6, this process also appears to depend on the ratio of C18:2*n*-6 to saturated fatty acid (Garg *et al.* 1989).

The levels of C12:0, C14:0 and C18:0, whether expressed in terms of mg/100 g muscle or as a proportion of the total fatty acids were not influenced by diet. However, C16:0 constituted a significantly higher proportion of total fatty acids in lambs offered the control and fish oil diets. The control and fish oil diets had the highest dietary C16:0 content due to the inclusion of Megalac a palm oil calcium soap high in C16:0 and in the latter diet fish oil which contains saturated straight chain fatty acids of which C16:0 is a major fatty acid (Enser, 1991). In contrast diets containing fish meal or protected fish oil had no effect on the proportion of C16:0 in muscle fatty acids (Mills *et al.* 1992 and Ashes *et al.* 1992b), although Mandell *et al.* (1997) reported an increase in tissue C16:0 concentration after feeding fish meal. It is possible that feeding lambs the linseed and linfish diet decreased fatty acid synthesis *de novo* and consequently reduced the concentration of C16:0, the major end product as well as C18:1*n*-7 its elongation/desaturation product. St John *et al.* (1987) and Rule *et al.* (1989), observed a decrease in C16:0 in the adipose tissue of steers fed diets supplemented with rapeseed, but did not demonstrate an increase in C18:1*n*-7 concentration which is similar to observations in the current study. However, Rule *et al.* (1989) observed a greater deposition of C18:0 after feeding diets supplemented with rapeseed as in the current study.

The levels of C18:1*n*-9 were lower in lambs offered diets containing fish oil than in those offered the control diet. Ashes et *al.* (1992b) demonstrated that increases in longer chain *n*-3 PUFA in muscle phospholipid occurred at the expense of *n*-6 PUFA or *n*-9 monounsaturated fatty acids. This may explain observations in the current study where significant decreases in C18:2*n*-6, C20:4*n*-6 and C22:4*n*-6 fatty acids were observed in the intramuscular lipids of lambs offered diets containing *n*-3 PUFA supplements compared to control lambs.

The large increases in *trans* 11-C18:1 (vaccenic acid) deposition on feeding PUFA supplements reflect a high levels of incomplete rumen biohydrogenation of C18 unsaturated fatty acids and consequently the high level of *trans* C18:1 available for absorption at the duodenum. Similar observations were reported in steers (Scollan *et al.* 1997), and milk from dairy cattle offered diets containing fish oil (Mansbridge and Blake, 1997). In the previous experiment (Chapter 3), rumen disturbances were greater in lambs offered the diets containing linseed, fish oil and linfish diets. An increased content of *trans* C18:1 may also explain the lower levels of C18:0 in intramuscular lipids from lambs offered diets supplemented with *n*-3 PUFA sources compared to control fed lambs. Whilst *trans* fatty acids have been associated

with an increased risk in cardiovascular diseases, recent epidemiological evidence suggests that ruminant *trans* fatty acids, mainly *trans* vaccenic acid may not be a risk (Willet *et al.* 1993).

Soay lambs offered the control diet had higher intramuscular concentrations of PUFA than either the Suffolk or Friesland lambs on a similar diet. Since total intramuscular fat was not different between the three breeds, this suggests a more efficient conservation, uptake and incorporation of PUFA by the Soay breed. However upon supplementation with *n*-3 PUFA sources, most of these differences were eliminated with the Suffolk lambs having the largest increase in C22:6*n*-3 deposition when offered the fish oil diet. St. John *et al.* (1991) suggested that rates of desaturation and elongation were correlated with rates of fatty acid synthesis such that breeds with a higher propensity to gain weight as fat also exhibit greater rates of fatty acid desaturation and elongation. Such suggestions may explain the differences observed between breeds in the current study. Other possible explanations would be that the requirement for essential fatty acids (EFA) for growth, other than for muscle deposition was higher in Suffolk and Friesland than in Soay lambs. As such most of the dietary EFA escaping rumen biohydrogenation while feeding the control diet were utilised for growth and less was retained for synthesis and deposition into muscle lipids (Becker and Bruce, 1986).

Overall dietary factors were more effective than genetic factors in determining the fatty acid composition of tissues. However, the deposition of EFA such as C18:2*n*-6 into muscle lipids can also be affected by a host of factors other than overall general composition of the diet. Evidence suggests that exposure of ruminant animal to high environmental temperatures results in substantial alterations to the plasma and tissue C18:2*n*-6 levels (Noble *et al.* 1973). Seasonal variations in plasma C18:2*n*-6 concentrations have also been observed (O'Kelly, 1972). The magnitude of this effect is normally associated with genetic differences because the effect is more apparent in breeds of cattle from temperate zones (*Bos taurus*) than in Zebu cattle (*Bos indicus*) (O'Kelly, 1968). At similar environmental temperatures, Zebu cattle display higher plasma levels of C18:2*n*-6 than the temperate breeds. Given that the Soay breed is primitive like the Zebu cattle, while the Suffolk and Friesland are similar to *Bos taurus* cattle, the higher level of C18:2*n*-6 in Soay lambs, when offered the control diet maybe interpreted as a real genetic difference. Noble and Alderson (1992) reported similar findings between the lipid composition of tissues from Hebridean and Suffolk sheep. The fact that the Hebridean sheep in

the Noble and Alderson, (1992) study were kept under "alien" lowland intensive conditions under which ruminal biohydrogenation capacities should be maximal suggest that inherent differences in specific rumen function existed with consequential effects on carcass lipid and fatty acid quality. But in the absence of knowledge of the factors regulating fatty acid composition of ruminant muscle lipids, there is no readily available explanation of the observed differences.

4.7 CONCLUSIONS

The inclusion of fish oil in lamb diets depressed food intake and growth rate. Compared with the control fat (Megalac), the level of C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 in lamb muscle was significantly enhanced by linseed, while C20:5*n*-3 and C22:6*n*-3 levels were increased by fish oil, with proportional increases in the mixed diet. The level of C20:5*n*-3 and C22:6*n*-3 incorporation into muscle lipids was similar between Suffolk, Soay and Friesland breeds, but C18:3*n*-3 was significantly lower in Friesland lambs. Soay lambs had significantly higher levels of total *n*-3 polyunsaturated fatty acids compared to the other breeds when fed the control ration.

CHAPTER 5

THE EFFECTS OF DIETARY FAT SOURCE, BREED AND VITAMIN E SUPPLEMENTATION ON THE FATTY ACID COMPOSITION AND VITAMIN E CONTENT OF LAMB TISSUES

5.1 INTRODUCTION

In the previous studies (Chapter 3 and 4), whole linseed was not very effective in protecting C18:3*n*-3 from ruminal biohydrogenation, but offering lambs diets containing whole linseed increased C18:3*n*-3 and long chain C20 and C22 *n*-3 fatty acids in the intramuscular lipids. Although fish oil fatty acids were less susceptible to biohydrogenation and consequently increased long chain *n*-3 polyunsaturated fatty acids (PUFA) in the intramuscular lipids, they depressed food intake and growth rate in lambs. As such linseed was considered to have the best potential to improve the nutritional quality of lamb.

Supplementing ruminant diets with chemically protected lipids can prevent rumen biohydrogenation (Gulati *et al.* 1997). For example feeding lambs diets containing seeds encapsulated in a matrix of formaldehyde treated protein elevated tissue PUFA compared to feeding whole seeds (Ackerson and Johnson, 1975). The elevation of tissue polyunsaturation may increase the susceptibility of tissues to oxidation (Pearson and Gray, 1983). In ruminants, supra nutritional vitamin E elevates muscle and plasma vitamin E and delays oxidative changes post mortem (Wulf *et al.* 1995).

The objectives of this study were to investigate the effects of formaldehyde treated whole linseed with or without fish oil, and dietary vitamin E level on tissue fatty acid composition, vitamin E content and performance in two genotypes from contrasting production systems in the UK.

5.2 MATERIAL AND METHODS

5.2.1 Experimental animals, treatments and design

48 Suffolk x Lleyn and 48 Scottish Blackface ram lambs, with initial live weights of 24 and 18 kg, respectively were used in the study.

All diets were based on dried grass, and were formulated to be iso-energetic, iso-nitrogenous and to provide a similar level of fat (60 g/kg DM), from different fat sources (Table 5.1). The control diet contained Megalac, a saturated fat similar to that used in experiments one and two (Chapters 3 and 4). The second diet contained formaldehyde treated whole linseed as the fat source, and the third diet contained equal quantities (50:50), on a fat basis of formaldehyde treated whole linseed and fish oil. Whole linseed was encapsulated in a matrix of formaldehyde at the rate of 4 g/kg seed (Trouw Nutrition UK, Cheshire). Vitamin E was included as α-tocopherol acetate (Roche Products Limited) at 100 and 500 mg/kg, for the low and high vitamin E diets, respectively.

The six dietary treatments were therefore: Megalac with low vitamin E, (**ML**); Megalac with high vitamin E (**MH**); Protected linseed with low vitamin E (**LL**); Protected linseed with high vitamin E (**LH**); Protected linseed plus fish oil with low vitamin E (**LFL**); Protected linseed plus fish oil with high vitamin E (**LFH**). Lambs were allocated by breed and live weight to one of the six dietary treatments in a 3x2x2 factorial experimental design

5.2.2 Procedure and measurements

Lambs were individually housed in raised floor pens, and gradually adapted to a mixed diet containing equal quantities of the three experimental diets containing low vitamin E (ML, LL and LFL). After two weeks on the adaptation diet, all lambs were offered their respective dietary treatments. Feed was offered *ad libtum* and daily refusals recorded between 0800 and 0900 hours on Mondays, Wednesdays and Friday of each week. Feeding levels were adjusted to 115 %, of the previous recorded feed consumption. Feed samples were taken every fortnight and stored at 4°C prior to subsequent analysis for dry matter, organic matter, crude protein, neutral detergent fibre and total fat as described in section 2.1.1, 2.1.2, 2.1.3.1, 2.1.4 and 2.6.2, respectively of the Material and Methods (Chapter 2). Dietary vitamin E and selenium were analysed by Aspland and James Limited, Consultant Analysts, Cambridgeshire and ADAS

Laboratories, Wolverhampton, respectively.

Weekly live weights were recorded (between 1400 and 1500 hours), on Wednesday and Thursday for half the lambs on each treatment. Blood samples (7 ml x 2) were taken from all lambs via the jugular vein between 1100 and 1200 hours, into heparin tubes after 0, 5 and 8 weeks on the treatment diets. Samples were then immediately centrifuged at 2000 *g*, before removing the plasma and storing at minus 30°C prior to subsequent analysis for plasma vitamin E using the method of Burton *et al.* (1985), as described in section 2.71 of Material and Methods (Chapter 2).

At approximately half the potential mature live weight for each breed, animals were slaughtered and carcasses scored for conformation and fat class as described in section 2.5.2 of Material and Methods. This was on average 46 kg for the Suffolk and 36 kg for the Scottish Blackface lambs. Loin adipose tissue (LAT) samples, *semimembranosus* (SM) and *longissimus dorsi* (LD) muscle samples were taken as described in section 2.5.3 of the Materials and Methods. All samples were vacuum packed and frozen at minus 20°C, prior to fatty acid (LAT and SM) and vitamin E (LD) analysis as described in sections 2.6.3, 2.6.4 and 2.7.2, respectively of the Materials and Methods (Chapter 2).

5.2.3 Statistical analysis

Live weight gain for each animal was estimated from the regression of live weight on time. Average daily food intake expressed on a dry matter basis was calculated from the total food intake over the experimental period for each animal. The two parameters were expressed on a metabolic live weight ($W^{0.75}$) basis, to avoid confounding effects caused by breed differences in body weight. The visual scores, for carcass fatness and conformation were converted to numerical values using the European Union 15 point carcass fat and conformation scheme, prior to statistical analysis. All data was subjected to analysis of variance using a factorial randomised block design with the initial weight as a covariant where appropriate (Genstat 5; Lawes Agricultural Trust, 1995).

Table 5.1. *Raw materials and chemical composition of diets containing different fat sources and vitamin E levels*

Fat source	Megalac		Linseed		Linfish	
Vitamin E level	Low	High	Low	High	Low	High
Ingredient (g/kg)						
Dried grass	759	759	739	739	754	754
Sugar beet pulp (molassed)	105	105	105	105	105	105
Megalac	35	35	-	-	-	-
Protected whole linseed	-	-	85	85	42	42
Fish oil	-	-	-	-	15	15
Soyabean meal	46	46	16	16	29	29
Molasses	25	25	25	25	25	25
Mineral and Vitamin premix	20	20	20	20	20	20
Ammonium chloride	5	5	5	5	5	5
Salt	5	5	5	5	5	5
Vitamin E mg/kg	100	500	100	500	100	500
Chemical composition (g/kg DM)						
Dry matter	942	929	935	929	935	935
Organic matter	884	880	891	889	886	891
Crude protein	174	170	172	172	170	174
Neutral detergent fibre	479	477	491	463	478	470
Total fat	45	50	50	51	51	56
Vitamin E (mg/kg DM)	137	453	129	423	97	411
Selenium (mg per kg DM)	1.04	0.93	0.93	0.79	0.96	0.87

5.3 RESULTS

5.3.1 Diet composition

Analysed chemical composition (Table 5.1), was approximately (g/kg DM); 887 organic matter, 172 crude protein and 476 neutral detergent fibre. Total fat ranged between 45-56 g/kg DM. Dietary α-tocopherol ranged between 97-137 mg/kg DM for the ML, LL and LFL diets and between 411-453 mg/kg DM for MH, LH and LFH diets. Selenium levels were approximately 1.0 mg/kg DM.

Dietary fatty acid composition is presented in Table 5.2. The fatty acid composition reflected dietary fat sources. The control diet contained high amounts of palmitic (C16:0) and oleic (C18:1*n*-9) acids, while α-linolenic acid (C18:3*n*-3) concentration was highest in diets containing formaldehyde treated whole linseed. The inclusion of fish oil in the linfish diet, increased the concentration of eicosapentaenoic (C20:5*n*-3) and docosahexaenoic (C22:6*n*-3) acids.

5.3.2 Diet, breed and vitamin E effects on animal performance

Animal performance data is summarised in Table 5.3a, and the significance of the main effects and interactions in Tables 5.3b. Dietary fat did not significantly influence daily food intake which was 144.0, 139.9 and 137.9 g/kg $W^{0.75}$ for animals offered the Megalac, linseed and linfish diets, respectively. In contrast, daily food intake was significantly higher in Suffolk lambs than in the Scottish Blackface with mean values of 151.4 and 129.8 g/kg $W^{0.75}$, respectively, (sed = 5.04, $p<0.001$).

Dietary vitamin E level did not significantly influence lamb performance. However, lambs offered diets containing Megalac ate significantly more of the Megalac high vitamin E (MH) compared to the Megalac low vitamin E (ML) diet. Mean daily food intakes were (g/kg $W^{0.75}$), 148.3 (MH) and 139.6 (ML), (sed = 4.00, $p<0.05$). Dietary fat, breed or vitamin E had no significant effect on daily live weight gain. Feed conversion ratio (FCR, kg feed/kg gain) was lower in the Scottish Blackface compared to Suffolk lambs with mean values of 6.7 and 7.9, respectively (sed = 0.412, $p<0.05$).

Table 5.2. *Fatty acid composition and total fatty acid intake of diets containing different fat sources and vitamin E levels*

	g/kg DM					
	Megalac		Linseed		Linfish	
	Low	High	Low	High	Low	High
Fatty acid						
C12:0 lauric	0.8	1.0	0.2	0.4	0.3	0.2
C14:0 myristic	0.6	0.7	0.2	0.3	1.3	1.4
C16:0 palmitic	15.1	16.5	5.5	6.6	6.8	7.8
C16:1 palmitoleic	0.4	0.4	0.3	0.3	1.5	1.6
C18:0 stearic	1.6	1.8	1.8	2.0	1.7	1.9
C18:1 trans	-	-	-	-	-	-
C18:1 n-9 oleic	10.4	11.7	6.8	7.9	6.1	6.7
C18:1 n-7 vaccenic	0.3	0.4	0.3	0.4	0.6	0.7
C18:2 n-6 linoleic	6.3	6.9	7.2	7.9	6.4	7.4
C18:3 n-3 α-linolenic	7.3	8.2	25.7	23.5	18.3	20.3
C20:5 n-3 eicosapentaenoic	-	-	-	-	2.2	2.4
C22:6 n-3 docosahexaenoic	-	-	-	-	1.1	1.2
Remaining fatty acids	1.4	1.5	1.4	1.1	2.5	2.2
Total Fat	45.2	49.9	50.1	51.1	51.1	56.0

5.3.3 Diet, breed and vitamin E effects on carcass weight and classification characteristics

Carcass weights and classification characteristics are summarised in Table 5.3a, and the significance of the main effects and interactions in Table 5.3b. Lambs offered diets containing fish oil had higher carcass fat scores when compared to lambs on the Megalac or linseed diets. Mean fat scores were 6.8, 7.1 and 8.2 for lambs fed the Megalac, linseed and linfish diets, respectively (sed = 0.41, $p<0.01$). Lambs offered diets containing *n*-3 PUFA sources (linseed and linfish diets), had lower carcass conformation scores than lambs offered the Megalac diet. Mean conformation scores were 7.3, 6.6 and 6.6 for Megalac, linseed and linfish diets, respectively (sed = 0.33, $p<0.05$).

Suffolk lambs had significantly heavier carcass weights, higher conformation and fat scores than Scottish Blackface (Table 5.3a). Mean values for Suffolk (Scottish Blackface in brackets) for hot carcass weight (kg) were 19.5 (14.2), cold carcass weight (kg) 18.8 (13.7), conformation scores 8.1 (5.6) and fat scores 8.1 (6.7), (sed = 0.40, 0.32, 0.27 and 0.33, respectively, $p<0.001$). Lambs offered any of the high vitamin E diets (MH, LH and LFH), tended to have heavier carcass weights compared to lambs on the low vitamin E diets (ML, LL and LFL), although the effect was not statistically significant. Mean values (kg) for hot carcass weight were 16.6 and 17.2 and cold carcass weights were 16.0 and 16.6, for lambs offered the low and high vitamin E diets, respectively (Table 5.3a).

Table 5.3a. *Effect of dietary fat, vitamin E and breed on the performance, carcass weights and classification of sheep*

	Megalac				Linseed				Linfish				
	Low		High		Low		High		Low		High		
	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
Animal performance													
Intake (g DM per kg $W^{0.75}$)	150.8	128.4	157.1	139.5	155.1	126.9	148.3	129.4	150.7	131.6	146.4	123.0	6.37
DLWG (g per kg $W^{0.75}$)	20.1	19.1	20.5	21.3	19.2	19.3	19.1	18.1	19.1	20.7	19.0	20.5	1.69
FCR (kg feed per kg gain)	7.6	6.9	7.7	6.6	8.2	6.8	7.9	7.3	8.1	6.6	7.9	6.1	0.57
Carcass weights													
Hot Carcass wt. (kg)	19.7	14.1	20.6	14.6	19.2	13.4	19.8	14.3	18.9	13.9	19.0	14.8	0.80
Cold Carcass wt (kg)	19.1	13.8	19.9	14.1	18.5	13.1	19.1	13.8	18.1	13.4	18.4	14.2	0.78
Carcass pH 24 hours	5.9	5.9	6.0	6.1	5.9	6.0	6.0	5.9	5.9	5.9	5.8	6.0	0.11
Carcass classification													
Conformation score	7.9	6.0	8.8	6.6	8.8	5.0	8.0	4.8	7.4	5.2	7.6	6.0	0.65
Fat score	7.4	6.5	7.4	6.0	7.5	6.0	9.0	5.9	9.3	7.7	8.0	8.0	0.81

Table 5.3b. *Effect of dietary fat, vitamin E and breed on the performance, carcass weights and classification of sheep (significance of main effects and interactions)*

	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Viatmin E
	sign	sign	sign	sign	sign	sign	sign
Animal performance							
Intake (g DM per kg	ns	***	ns	ns	*	ns	ns
$W^{0.75}$)	ns	ns	ns	ns	ns	ns	ns
DLWG (g per kg $W^{0.75}$)	ns	**	ns	ns	ns	ns	ns
FCR (kg feed per kg							
gain)							
Carcass weights							
Hot Carcass wt. (kg)	ns	***	ns	ns	ns	ns	ns
Cold Carcass wt (kg)	ns	***	ns	ns	ns	ns	ns
Carcass pH (24 hours)	ns	ns	ns	ns	ns	ns	
Carcass classification							
Conformation score	*	***	ns	*	ns	ns	ns
Fat score	**	***	ns	ns	ns	ns	ns

5.4 MUSCLE FATTY ACIDS

5.4.1 Diet, breed and vitamin E effects on *semimembranosus* muscle fatty acids

The weight (mg) of fatty acids per 100 g *semimembranosus* muscle (fatty acids content) are given in Tables 5.4a and the significance of the main effects and interactions in Table 5.4b. The percentage contribution of each fatty acid to total fatty acids (fatty acid composition) is shown in Table 5.5a and the significance of the main effects and interactions in Table 5.5b.

The total fatty acid weight (muscle lipid content), was higher in lambs offered either the linseed or linfish diets compared to lambs on the Megalac diet, reflecting their greater carcass fatness (Table 5.4a). Mean values of total fatty acids were 2.3, 3.1 and 3.0 % by weight of muscle, for lambs offered the Megalac, linseed and linfish diets, respectively (sed = 1.73, $p<0.001$). As such, individual fatty acids generally made a greater contribution to muscle weight in lambs fed on the linseed and linfish diets than in lambs on the Megalac diet, although all *n*-6 PUFA were higher in lambs on the Megalac diet.

5.4.1.1 Main effects of dietary fat, breed and vitamin E on muscle fatty acid profiles

Main effects of dietary fat, breed and vitamin E level on *semimembranosus* muscle fatty acids composition is presented graphically in Figures 5.1, 5.2 and 5.3, respectively.

(a) *n*-3 fatty acids

Intramuscular lipids from lambs offered the linseed diet, contained the highest level of C18:3*n*-3. The mean values were 49.3, 113.4 and 71.6 mg/100 g muscle (sed = 6.93, $p<0.001$, Table 5.4a and Figure 5.1), or 2.1, 3.7 and 2.4 % (sed = 0.17, $p<0.001$, Table 5.5a), for lambs offered the Megalac, linseed and linfish diets, respectively. The concentration of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3, was highest in lambs offered the linfish diet. Mean values for C20:5*n*-3 (24.6, 37.8 and 52.5), 22:5*n*-3 (24.7, 26.9 and 43.2) and C22:6*n*-3 (7.6, 7.8 and 13.5) mg/100 g muscle (sed = 1.90, 2.25 and 0.59, respectively, $p<0.001$, Table 5.4a and Figure 5.1) for lambs offered the Megalac, linseed and linfish diets, respectively. Mean values on a percentage basis were C20:5*n*-3 (1.1, 1.3 and 1.8), C22:5*n*-3 (1.1, 1.2 and 1.5) and C22:6*n*-3 (0.3, 0.3 and 0.5), (sed = 0.09, 0.07 and 0.03, $p<0.001$, Table 5.5a) for lambs offered the Megalac, linseed and linfish diets, respectively

Suffolk lambs contained significantly higher concentrations of C18:3*n*-3 than the Scottish

Blackface (mean values were 86.9 *vs* 69.3 mg/100 g muscle (sed = 5.66, p<0.01, Table 5.4a and Figure 5.2) and 3.0 *vs* 2.58 %, (sed = 0.143, p<0.01, Table 5.5a) for Suffolk and Scottish Blackface lambs, respectively. However, Scottish Blackface lambs had the highest concentration of C20:5*n*-3 (mean value were 35.8 and 40.9 mg/100 g muscle for Suffolk and Scottish Blackface lambs, respectively, sed = 1.56, p<0.01, Table 5.4a and Figure 5.2).

All lambs offered diets with high vitamin E levels (MH, LH and LFH), contained significantly higher concentrations of all *n*-3 PUFA compared to lambs on the low vitamin E diets (ML, LL and LFL) when expressed on a weight basis (mg/100 g muscle). Mean values for low vitamin E (high vitamin E in brackets) were; 72.5 (83.7), 36.4 (40.3) and 9.1 (10.2) mg/100 g muscle for C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 fatty acids, respectively (sed = 5.66, 1.56 and 0.48, respectively, p<0.05, Table 5.4a and Figure 5.3). Among the three fatty acids, the concentration of C18:3*n*-3 was higher in high compared to low vitamin E diets, when expressed as a percentage of total fatty acids (mean values were 2.6 and 2.9 %, for high and low vitamin E diets, respectively, sed = 0.14, p< 0.01, Table 5.5a).

(b) *n*-6 fatty acids

Lambs on the linseed diet contained the highest level of C18:2*n*-6 when expressed on a weight basis but the contribution of C18:2*n*-6 to total fatty acids was highest in lambs offered the Megalac diet. Mean values were 97.8, 100.8 and 82.6 mg/100 g muscle (sed = 5.03, p< 0.001, Table 5.4a and Figure 5.1) and 4.3, 3.4 and 2.8 % (sed = 0.19, p<0.001, Table 5.5a) for lambs fed the Megalac, linseed and linfish diets, respectively. Likewise, the concentration of C20:4*n*-6 was highest in lambs fed the Megalac diet with mean values of 35.0, 29.6 and 22.5 mg/100 g muscle (sed = 1.51, p<0.001, Table 5.4a and Figure 5.1), for lambs fed the Megalac, linseed and linfish diets, respectively. Mean values on a percentage basis for C20:4*n*-6 were 1.6, 1.0 and 0.8 % (sed = 0.08, p<0.001, Table 5.5a), for lambs fed the Megalac, linseed and linfish diets, respectively

Suffolk lambs contained significantly higher concentrations of C18:2*n*-6 than Scottish Blackface, when expressed on a weight basis (mg/100 g muscle), but the difference was not apparent when the fatty acids were expressed on a percentage basis. Mean values for C18:2*n*-6 were 101.7 *vs* 85.7 mg/100 g muscle, sed = 4.11 p<0.01, Table 5.4a and Figure 5.2) and 3.6 *vs* 3.5 %, sed = 0.153, (Table 5.5a and 5.5b) for Suffolk and Scottish Blackface lambs,

respectively.

All lambs offered diets with a high level of vitamin E (MH, LH and LFH) contained significantly higher concentration of C18:2*n*-6 than lambs fed diets with a low level of vitamin E (ML, LL and LFL). Mean values were 99.8 *vs* 87.7 mg/100 g muscle (sed = 4.11, $p<0.01$, Table 5.4a and Figure 5.3) and 3.7 *vs* 3.3 % (sed = 0.15, $p<0.01$, 5.5a), for high and low vitamin E diets, respectively.

(c) Monoenoic and saturated fatty acids

The major monoenoic fatty acid, C18:1*n*-9 was significantly lower in lambs offered the Megalac (control) diet than in lambs fed either diets containing linseed (linseed and linfish diets), when the fatty acid was expressed on a weight basis (mg/100 g muscle) but the inverse was true when the acid was expressed as a percentage of total fatty acids. Mean values were 876, 1136 and 1029 mg/100 g muscle (sed = 88.9, $p<0.05$, Table 5.4a) and 37.5, 36.3 and 33.5 % (sed = 0.78, $p<0.05$, Table 5.5a) for lambs offered the Megalac, linseed and linfish diets, respectively.

Intramuscular lipids from lambs offered the linseed and linfish diets contained the highest level of C18:0. The mean values were 297, 416 and 410 mg/100 g (sed = 30.0, $p<0.001$, Table 5.4a) or 12.7, 13.4 and 13.5 % (sed = 0.29, $p<0.05$, Table 5.5a and 5.5b), for lambs offered the Megalac, linseed and linfish diets, respectively). Similarly, lambs fed either diets with linseed (linseed and linfish diets) contained significantly higher levels of C14:0 and C16:0, than control lambs, when the fatty acids were expressed on a weight basis (mg/100 g muscle) but not when expressed as a percentage of total fatty acids (Table 5.5a). Mean values (mg/100 g muscle) were C14:0 (56.9, 73.1 and 71.8, sed = 6.22, $p<0.05$), and C16:0 (531, 701 and 710, sed = 55.4, $p<0.01$) for Megalac, linseed and linfish diets, respectively (Table 5.4a).

Compared to Scottish Blackface, intramuscular lipids of Suffolk lambs contained a higher level of C12:0 and C14:0 fatty acids. The mean values for Suffolk (Scottish Blackface in brackets) were C12:0, 7.1 (5.1) and C14:0, 74.6 (59.9) mg/100 g muscle (sed = 0.58 and 5.08; $p<0.001$ and $p<0.01$, respectively, Table 5.4a). Suffolk lambs also contained the highest proportion of C16:0 with a mean value of 23.1 compared to 22.3 % in Scottish Blackface lambs (sed = 0.33, $p< 0.05$, Table 5.5a).

(d) Trans fatty acids

Lambs offered the linfish diet contained double the concentration of *trans* C18:1 compared with lambs offered either the Megalac or linseed diets. Mean value were 41.1, 58.1 and 101.1 mg/100 g muscle (sed = 8.28, $p<0.001$, Table 5.4a) and 1.7, 1.9 and 3.4 % (sed = 0.25, $p<0.001$, Table 5.5a), for Megalac, linseed and linfish diets, respectively. Lambs offered the linseed diet also contained significantly higher concentrations of *trans* C18:1 than lambs fed the Megalac diet.

5.4.1.2 Interaction effects

Scottish Blackface lambs offered the Megalac diet contained a higher proportion of C18:2*n*-6 in the intramuscular lipids than Suffolk lambs fed the same diet, whilst the opposite was true when Scottish Blackface and Suffolk lambs were offered the linseed diet. Mean values for C18:2*n*-6 in lambs offered the Megalac diet (4.0 *vs* 4.7 %) or linseed diet (3.7 *vs* 3.1 %), (sed = 0.27, $p<0.01$, Table 5.5a), for Suffolk and Scottish Blackface lambs, respectively. There was a trend towards increased C20:5*n*-3 concentrations in Scottish Blackface lambs compared to Suffolk lambs when offered the linfish diet (57.7 *vs* 47.4 mg/100 g muscle, respectively sed =2.69, $p = 0.06$). Similarly, intramuscular concentrations of C20:5*n*-3 tended to be higher in Scottish Blackface lambs offered diets with a high vitamin E level, compared to Suffolk lambs on the same diets (36.4 and 44.1 mg/100 g muscle (sed = 2.20, $p = 0.09$, Table 5.5a), for Suffolk and Scottish Blackface, respectively).

The proportion of C22:6*n*-3 in the total fat was higher in Scottish Blackface than Suffolk lambs when the two breeds were offered high vitamin E diets (MH, LH or LFH) (mean values were 0.3 *vs* 0.4 % (sed = 0.045, $p<0.05$, Table 5.5a) for Suffolk and Scottish Blackface lambs, respectively). Although not statistically significant, Suffolk lambs offered the linfish diet with a low vitamin E level (LFL) contained higher levels of *trans* C18:1 than Scottish Blackface lambs on the same diet. The mean values for Suffolk lambs were 109.4, compared to 81.1 mg/100 g muscle in Scottish Blackface lambs, sed = 16.55, $p = 0.09$, (Table 5.4a). In contrast, Scottish Blackface lambs offered the linfish diet with a high vitamin E level (LFH) contained higher intramuscular levels of *trans* C18:1, than Suffolk lambs on the same diet. The mean value for Scottish Blackface lambs was 118.0 compared to 95.9 mg/100 g muscle in Suffolk lambs, (Table 5.4a).

Table 5.4a. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the semimembranosus muscle of sheep*

	mg per 100 g muscle												
	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
Fatty acid	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
C12:0 lauric	6.3	4.4	7.9	3.0	7.2	4.7	7.3	7.2	6.9	5.1	7.1	6.3	1.43
C14:0 myristic	65.7	50.7	74.2	36.9	75.5	60.4	74.2	82.3	76.1	69.8	82.0	59.1	12.43
C16:0 palmitic	582	526	619	397	693	627	708	775	735	690	789	625	110.8
C16:1 palmitoleic	49.8	46.1	54.5	34.4	55.3	50.0	55.5	63.0	66.5	60.9	64.3	53.2	9.14
C18:0 stearic	325	312	327	224	406	391	402	466	408	419	446	367	59.9
C18:1 trans	46.9	35.8	57.9	23.8	62.6	48.0	60.3	61.7	109.4	81.1	95.9	118.0	16.55
C18:1 *n*-9 oleic	957	897	990	658	1113	1041	1145	1246	1096	1002	1127	889	177.8
C18:1 *n*-7 vaccenic	38.1	43.3	40.8	34.2	38.5	40.9	44.3	50.3	50.0	49.5	48.3	42.0	6.91
C18:2 *n*-6 linoleic	95.5	92.6	107.4	95.6	99.5	78.3	123.0	102.6	89.6	70.5	95.5	75.0	10.07
C18:3 *n*-3 linolenic	52.7	41.3	61.0	42.4	120.6	84.4	124.8	123.6	76.0	59.9	86.0	64.4	13.86
C20:3 *n*-6	3.4	4.4	3.9	3.9	3.1	3.2	3.5	3.6	4.2	4.0	3.6	3.8	0.39
C20:4 *n*-6 arachidonic	32.8	34.9	35.9	36.5	28.5	28.7	34.6	26.6	23.0	22.2	24.2	20.7	3.02
C20:4 *n*-3	0.5	1.5	2.5	0.8	1.9	1.8	1.6	3.2	10.2	14.8	11.9	16.0	1.95
C20:5 *n*-3 EPA[1]	23.5	24.5	24.3	26.3	36.4	34.5	35.8	44.5	45.7	53.7	49.2	61.6	3.81
C22:4 *n*-6	1.2	1.5	1.6	1.8	1.1	1.1	1.7	1.0	0.5	0.9	0.3	1.0	0.70
C22:5 *n*-3 DPA[2]	24.2	25.7	25.0	24.0	34.3	33.4	35.4	44.4	40.2	45.1	42.4	45.3	4.50
C22:6 *n*-3 DHA[3]	7.3	6.6	7.8	8.6	7.2	7.2	7.7	9.1	12.8	13.3	13.2	14.5	1.18
Remaining fatty acids	166	151	178	114	239	228	195	276	273	251	217	267	38.04
Total fatty acids	2517	2345	2660	1806	3060	2804	3101	3429	3162	2952	3241	2767	424.8

[1]EPA=Eicosapentaenoic acid; [2]DPA=Docosapentaenoic acid; [3]DHA=Docosahexaenoic acid

Table 5.4b. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the semimembranosus muscle of sheep (significance of main effects and interactions)*

	mg/100 g muscle						
	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
Fatty acid	sign	sign	sign	sign	sign	sign	sign
C12:0	ns	***	ns	ns	ns	ns	ns
C14:0	*	**	ns	ns	ns	ns	ns
C16:0	**	ns	ns	ns	ns	ns	ns
C16:1	**	ns	ns	ns	ns	ns	ns
C18:0	***	ns	ns	ns	ns	ns	ns
C18:1 trans	***	ns	ns	ns	ns	ns	ns
C18:1 *n*-9	*	ns	ns	ns	ns	ns	ns
C18:1 *n*-7	ns	ns	ns	ns	ns	ns	ns
C18:2 *n*-6	***	***	**	ns	ns	ns	ns
C18:3 *n*-3	***	**	*	ns	ns	ns	ns
C20:3 *n*-6	*	ns	ns	ns	ns	ns	ns
C20:4 *n*-6	***	ns	ns	ns	ns	ns	ns
C20:4 *n*-3	***	*	ns	*	ns	ns	ns
C20:5 *n*-3 EPA[1]	***	**	*	ns	ns	ns	ns
C22:4 *n*-6	*	ns	ns	ns	ns	ns	ns
C22:5 *n*-3 DPA[2]	***	ns	ns	ns	ns	ns	ns
C22:6 *n*-3 DHA[3]	***	ns	*	ns	ns	ns	ns
Total fatty acids	***	ns	ns	ns	ns	ns	ns

ns = not significant; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$

Table 5.5a. ***Effect of dietary fat, vitamin E and breed on the fatty acid composition of the semimembranosus muscle of sheep***

	% by weight of total fatty acids												
	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
Fatty acid	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
C12:0 lauric	0.26	0.19	0.29	0.17	0.24	0.17	0.24	0.21	0.23	0.17	0.22	0.25	0.046
C14:0 myristic	2.57	2.15	2.77	2.03	2.46	2.16	2.44	2.35	2.46	2.31	2.53	2.13	0.228
C16:0 palmitic	23.06	22.29	23.25	21.94	22.65	22.19	22.70	22.18	22.79	22.89	24.28	22.42	0.817
C16:1 palmitoleic	1.99	1.95	2.05	1.90	1.79	1.76	1.79	1.83	2.09	2.04	1.96	1.95	0.116
C18:0 stearic	12.85	13.22	12.31	12.38	13.38	13.93	12.88	13.55	12.88	14.25	13.79	13.09	0.584
C18:1 trans	1.79	1.51	2.14	1.32	2.00	1.71	2.03	1.80	3.61	2.71	2.99	4.35	0.495
C18:1 *n*-9 oleic	37.87	38.21	37.32	36.45	36.11	36.42	36.58	36.06	33.49	34.02	34.47	31.84	1.555
C18:1 *n*-7 vaccenic	1.55	1.83	1.55	1.90	1.28	1.47	1.42	1.48	1.57	1.70	1.48	1.55	0.112
C18:2 *n*-6 linoleic	3.91	4.02	4.07	5.34	3.36	3.01	4.05	3.18	3.15	2.49	2.94	2.80	0.375
C18:3 *n*-3 linolenic	2.13	1.80	2.26	2.35	3.97	3.18	4.09	3.73	2.62	2.05	2.69	2.35	0.349
C20:3 *n*-6	0.14	0.20	0.15	0.22	0.11	0.12	0.12	0.11	0.15	0.14	0.11	0.14	0.017
C20:4 *n*-6 arachidonic	1.37	1.53	1.36	2.04	0.98	1.12	1.15	0.89	0.88	0.83	0.75	0.77	0.160
C20:4 *n*-3	0.02	0.07	0.10	0.04	0.07	0.07	0.05	0.10	0.32	0.50	0.38	0.61	0.068
C20:5 *n*-3 EPA[1]	0.97	1.10	0.92	1.46	1.23	1.33	1.18	1.40	1.61	1.91	1.56	2.30	0.184
C22:4 *n*-6	0.05	0.07	0.06	0.10	0.03	0.04	0.06	0.03	0.01	0.02	0.01	0.04	0.026
C22:5 *n*-3 DPA[2]	1.00	1.13	0.95	1.34	1.15	1.25	1.14	1.33	1.38	1.60	1.33	1.68	0.148
C22:6 *n*-3 DHA[3]	0.31	0.29	0.30	0.48	0.25	0.29	0.26	0.31	0.46	0.49	0.42	0.54	0.064
Remaining fatty acids	6.59	6.43	6.60	6.27	7.64	8.28	6.43	8.05	8.89	8.39	6.93	9.77	0.815
Total fatty acids	93.92	94.07	93.88	94.22	92.93	92.33	94.03	92.50	91.77	92.24	93.60	90.98	0.754

[1]EPA=Eicosapentaenoic acid; [2]DPA=Docosapentaenoic acid; [3]DHA=Docosahexaenoic acid

Table 5.5b. ***Effect of dietary fat, vitamin E and breed on the fatty acid composition of the semimembranosus muscle of sheep (significance of main effects and interactions)***

	% by weight of total fatty acids						
	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
Fatty acid	sign	sign	sign	sign	sign	sign	sign
C12:0	ns	**	ns	ns	ns	ns	ns
C14:0	ns	***	ns	ns	ns	ns	ns
C16:0	ns	*	ns	ns	ns	ns	ns
C16:1	***	ns	ns	ns	ns	ns	ns
C18:0	*	ns	ns	ns	ns	ns	ns
C18:1 trans	***	ns	ns	ns	ns	ns	*
C18:1 *n*-9	***	ns	ns	ns	ns	ns	ns
C18:1 *n*-7	***	***	ns	ns	ns	ns	ns
C18:2 *n*-6	***	ns	**	**	ns	ns	ns
C18:3 *n*-3	***	**	*	ns	ns	ns	ns
C20:3 *n*-6	***	***	ns	***	ns	ns	ns
C20:4 *n*-6	***	ns	ns	**	ns	ns	*
C20:4 *n*-3	***	**	ns	**	ns	ns	ns
C20:5 *n*-3 EPA[1]	***	***	ns	ns	*	ns	ns
C22:4 *n*-6	***	ns	ns	ns	ns	ns	ns
C22:5 *n*-3 DPA[2]	***	***	ns	ns	ns	ns	ns
C22:6 *n*-3 DHA[3]	***	*	ns	ns	*	ns	ns
Total fatty acids	***	*	ns	ns	*	ns	ns

ns = not significant; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$

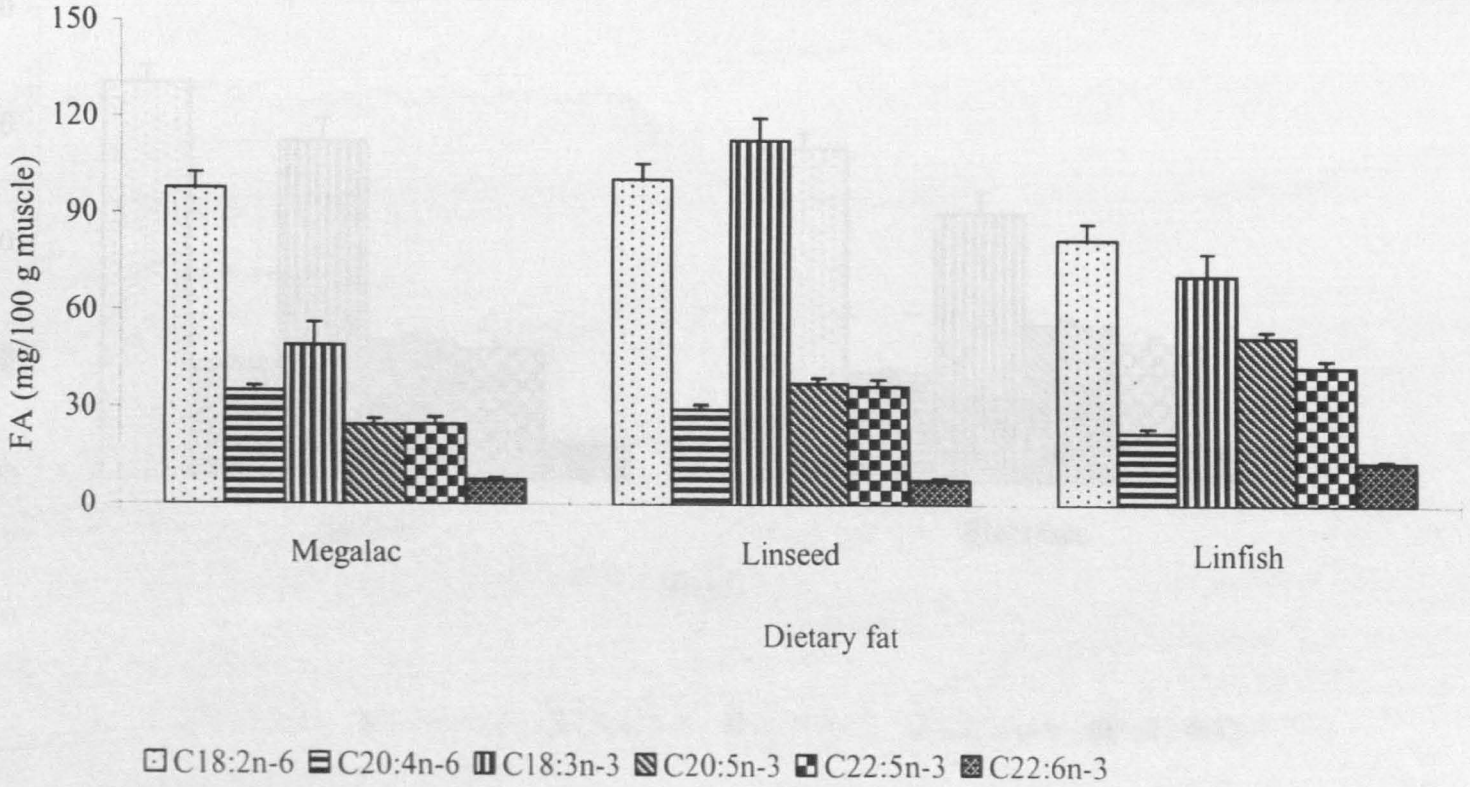


Figure 5.1 *Fatty acid content of selected PUFA in the semimembranosus of lambs fed different dietary fats*

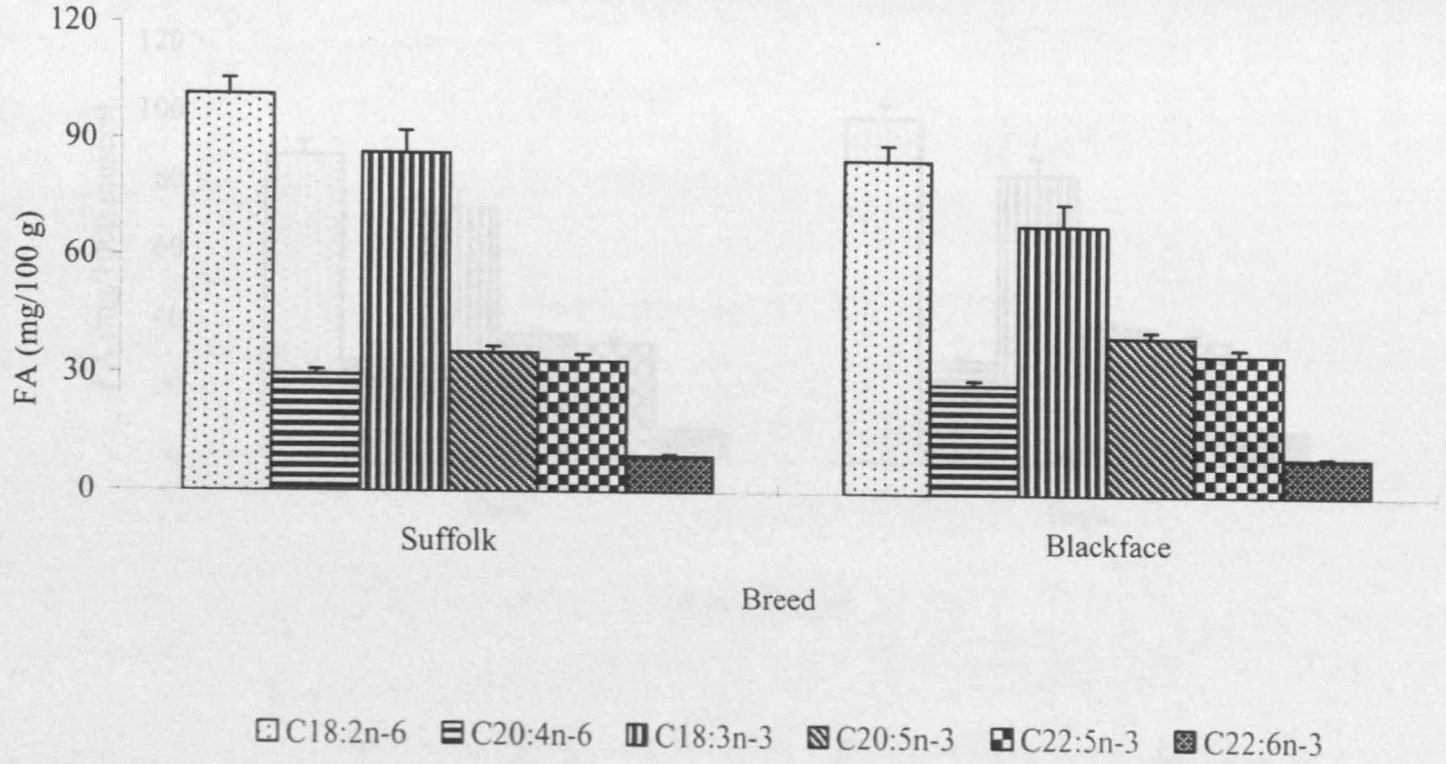


Figure 5.2 *Breed effects on content of selected PUFA in the semimembranosus muscle of lambs*

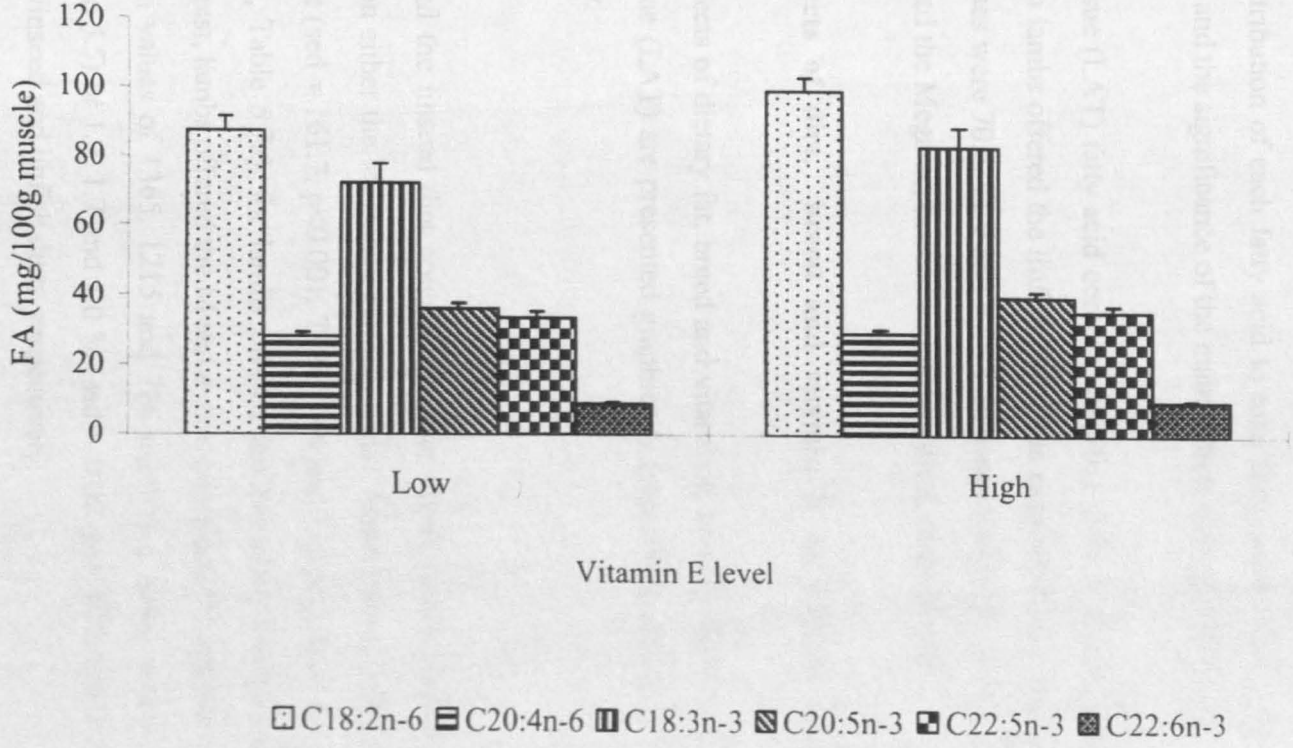


Figure 5.3 *Effect of vitamin E supplementation upon content of selected PUFA in the semimembranosus of lamb*

5.5 ADIPOSE TISSUE FATTY ACID COMPOSITION

5.5.1 Diet, breed and vitamin E effects on loin adipose tissue fatty acids

The weight (mg) of fatty acids per 100 g loin adipose tissue (fatty acids content) are given in Tables 5.6a and the significance of the main effects and interactions are in Table 5.6b. The percentage contribution of each fatty acid to total fatty acids (fatty acid composition) is shown in Table 5.7a and the significance of the main effects and interactions are in Table 5.7b.

The loin adipose tissue (LAT) fatty acid content was 70 ± 2 % of the tissue weight, but was significantly lower in lambs offered the linfish diet when expressed on a weight basis (mg/100 g tissue). Mean values were 70.6, 71.9 and 67.6 %of tissue weight (sed = 1.71, $p<0.05$, Table 5.6a) for lambs offered the Megalac, linseed and linfish diets, respectively.

5.5.1.1 Main effects of diet, breed and vitamin E on adipose tissue fatty acid composition

The main effects of dietary fat, breed and vitamin E level on fatty acid composition of the loin adipose tissue (LAT) are presented graphically (mg/100 g tissue) in Figures 5.5, 5.6 and 5.7, respectively.

(a) PUFA content

Lambs offered the linseed diet contained higher levels (more than 2x) of C18:3*n*-3, compared to lambs on either the Megalac or linfish diets. Mean values were, 1165, 3133 and 1338 mg/100 g tissue (sed = 161.7, $p<0.001$, Table 5.6a and Figure 5.5) or 1.7, 4.3 and 2.0 %, (sed =0.23, $p<0.001$, Table 5.7a), for lambs offered the Megalac, linseed and linfish diets, respectively. In contrast, lambs offered the Megalac diet contained the highest concentration of C18:2*n*-6, with mean values of 1365, 1215 and 726 mg/100 g tissue (sed = 63.8, $p<0.001$, Table 5.6a and Figure 5.5) or 1.9, 1.7 and 1.0 % (sed = 0.09, $p<0.001$, Table 5.7a), for lambs offered the Megalac, linseed and linfish diets, respectively.

Suffolk lambs contained higher concentrations of C18:2*n*-6 and C18:3*n*-3 than Scottish Blackface lambs. Mean values were C18:2*n*-6 (1158 *vs* 1046) and C18:3*n*-3 (2057 *vs* 1700) mg/100 g tissue, (sed = 52.1 and 132, $p<0.05$ and $p<0.01$, Table 5.6a and Figure 5.6), for Suffolk and Scottish Blackface lambs, respectively. When the fatty acids were expressed as a proportion of the total fatty acids, C18:3*n*-3 was significantly higher in Suffolk lambs than in

Scottish Blackface lambs (mean value were 2.9 *vs* 2.4 %, respectively, sed = 0.19, $p<0.05$, Table 5.7a).

Lambs offered diets with high levels of vitamin E (MH, LH and LFL) had a greater concentration of C18:2*n*-6 and C18:3*n*-3 in the adipose tissue. Mean values were C18:2*n*-6 (1203 *vs* 1001 mg/100 g tissue, sed = 52.1, $p<0.001$, Table 5.6a and Figure 5.7) or 1.7 *vs* 1.5 % (sed = 0.07, $p<0.01$, Table 5.7a) and mean values for C18:3*n*-3 (2047 *vs* 1710 mg/100 g tissue, sed = 132.0, $p<0.05$, Table 5.6a and Figure 5.7) or 2.9 *vs* 2.5 % (sed = 0.19, $p<0.05$, Table 5.7), for lambs offered high *vs* low vitamin E diets, respectively.

(b) Monoenoic and saturated fatty acids

Lambs offered the Megalac diet had a greater concentration of C18:1*n*-9 and C18:1*n*-7 (*cis* vaccenic acid) in the adipose tissue compared to lambs offered either the linseed or linfish diets. Mean values for C18:1*n*-9 were 21.2, 20.0 and 18.8 % of tissue weight (g/100 g tissue) (sed = 0.55, $p<0.001$, Table 5.6a) or 30.1, 27.8 and 28.0 % of total fatty acids (sed = 0.65, $p<0.01$, Table 5.7a) for lambs offered the Megalac, linseed and linfish diets, respectively.

Lambs offered the Megalac diet also contained more palmitoleic acid (C16:1) than lambs fed either the linseed or linfish diets. The mean values were 2065, 1729 and 1723 mg/100 g tissue, (sed = 65.4, $p< 0.001$, Table 5.6a) or 2.9, 2.5 and 2.6 % (sed = 0.083, $p<0.001$, Table 5.7a), for lambs offered the Megalac, linseed and linfish diets, respectively.

The content of C18:1*n*-9 was on average 20 % of the loin adipose tissue weight in both the Suffolk and Scottish Blackface lambs, but when the fatty acid was expressed as a proportion of the total fatty acids, Scottish Blackface lambs had a higher concentration than Suffolk lambs. The mean values were 29.3 and 28.0 %, respectively, (sed = 0.53, $p<0.05$, Table 5.7a). Similarly, Scottish Blackface lambs had greater concentrations of C18:1*n*-7 than Suffolk lambs (mean values were 585 *vs* 650 mg/00 g tissue (sed = 27.50, $p<0.001$, Table 5.6a) or 0.8 *vs* 0.9 % (sed = 0.03, $p<0.001$, Table 5.7a) for Suffolk and Scottish Blackface lambs, respectively).

Lambs offered the linfish diet had the highest concentration of stearic acid (C18:0). Mean values were 6540, 7420 and 8642 mg/100 g tissue (sed = 482.3, $p<0.001$, 5.6a) or 9.2, 10.4 and 12.8 % (sed = 0.66, $p<0.001$, Table 5.7a) for lambs offered the Megalac, linseed and linfish

diets, respectively. Similarly, the content of C16:0 when expressed on a percentage basis, was significantly higher in lambs offered the linfish diet, than in lambs either the Megalac or linseed diets. Mean values were 21.3, 20.4 and 23.5 % for lambs offered the Megalac, linseed and linfish diets, respectively (sed = 0.911, $p<0.01$, Table 5.7a). The concentrations of C10:0, C12:0 and C14:0 fatty acids was similar in lambs offered any of the diets, when expressed on a weight or percentage basis.

(c) Trans, branched and odd chain fatty acids

Lambs offered the linfish diet contained more than double the concentration of *trans* C18:1 than lambs offered either the Megalac or linseed diets. Mean values were 1037, 1205 and 2718 mg/100 g tissue (sed = 263, $p<0.001$, Table 5.6a) or 1.7, 1.5 and 4.0 % (sed = 0.34, $p<0.001$, Table 5.7a), for lambs on the Megalac, linseed and linfish diets, respectively.

In contrast, the adipose tissue from lambs offered the linfish diet contained the lowest concentration of odd chain (C15:0, C17:0) and branched chain fatty acids. Mean values were C15:0 (1283, 1029 and 660 mg/100 g tissue, sed = 88.6, $p<0.001$, Table 5.6a) or (1.8, 1.4 and 1.0 %, sed = 0.12, $p<0.001$, Table 5.7a) and for C17:0 (2964, 2812 and 1981 mg/100 g tissue, sed = 181.4, $p<0.001$, Table 5.6a) or (4.2, 3.9 and 3.0 %, sed = 0.25, $p<0.001$, Table 5.7a) for lambs offered the Megalac, linseed and linfish diet, respectively.

Mean values for branched chain fatty acids were 10.9, 9.8 and 5.4 % of tissue weight (sed = 1.02, $p<0.001$, Table 5.6a) or 15.6, 13.6 and 8.1 % of total fatty acids (sed = 1.44, $p<0.001$, Table 5.7a), for lambs offered the Megalac, linseed and linfish diets, respectively.

5.5.1.2 Interaction effects

Scottish Blackface lambs offered diets with low vitamin E levels (ML, LL and LFL) had greater concentrations of C18:1*n*-9 and C16:1, than Suffolk lambs fed the same diets ($p<0.001$). Mean values for C18:1*n*-9 were 18.9 and 20.7 % of tissue weight, for Suffolk and Scottish Blackface lambs on low vitamin E diets, respectively (sed = 0.64, $p<0.001$, Table 5.7a). The content of C18:1*n*-7 (*cis* vaccenic acid) was higher in Scottish Blackface lambs offered the Megalac diet, compared to Suffolk on the same diet. Mean values were 629.3 and 720.4 mg/100 g tissue for Suffolk and Scottish Blackface lambs on the Megalac diet, respectively (sed = 33.69, $p<0.05$, 5.6a). Saturated fatty acids (C12:0, C14:0 and C16:0) were

higher in Suffolk lambs offered diets with high vitamin E (MH, LH and LFH) than in Scottish Blackface lambs offered the same diets. Mean values were C12:0 (145.8 and 72.0 mg/100 g tissue), C14:0 (2.4 and 1.6 g/100 g tissue) and C16:0 (17.2 and 14.2 g/100 g tissue), (sed = 0.95, $p<0.05$, Table 5.7a) for Suffolk and Scottish Blackface lambs offered diets with high vitamin E, respectively.

Table 5.6a. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the loin adipose tissue of sheep*

	mg per 100 g tissue												
	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
Fatty acid	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
C10:0	183	168	283	121	204	145	198	150	229	179	181	155	33.2
C12:0	106	103	179	66	97	82	129	84	120	70	130	66	26.5
C14:0	2025	1958	2966	1586	1963	1832	2067	1677	2142	1638	2198	1627	280.4
C15:0	1296	1199	1324	1311	1094	894	1031	1096	695	703	665	576	177.1
C16:0	14353	14839	18838	12562	14296	15093	15468	14134	15833	14772	17193	15902	1649.4
C16:1	1870	2145	2260	1985	1603	1748	1789	1775	1690	1830	1625	1746	130.8
C17:0	2927	3008	2983	2938	2862	2414	2859	3115	1891	2210	2137	1685	362.7
C18:0	6428	6647	8100	4983	7189	6974	7932	7583	8203	8127	9421	8819	964.6
C18:1 trans	1015	869	1530	735	1071	1063	1631	1054	2956	1743	2188	3986	527.7
C18:1	19675	21858	23206	19969	18947	20787	20293	19775	17970	19549	18765	19046	1104.1
C18:1 n-7	582	741	676	700	486	595	661	542	575	591	529	728	47.6
C18:2 n-6	1271	1322	1540	1327	1109	947	1567	1236	732	626	731	817	127.5
C18:3 n-3	1155	1088	1413	1005	3121	2392	3912	3105	1352	1154	1388	1457	323.4
WBC[1]	11875	11138	8979	11499	10350	8805	9367	10542	5094	6950	4582	5031	2032.8
Unidentified	3875	3828	3949	3776	6105	6450	5716	6332	6290	6908	5191	8884	900.0
Total FA	**68635**	**70911**	**78225**	**64563**	**70496**	**70223**	**74620**	**72200**	**65772**	**67048**	**66925**	**70526**	**3412.5**

WBC[1]-weight of branched chain fatty acids

Table 5.6b. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the loin adipose tissue of sheep (significance of main effects and interactions)*

	mg per 100 g muscle						
	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
Fatty acid	sign	sign	sign	sign	sign	sign	sign
C10:0	ns	***	ns	ns	ns	ns	*
C12:0	ns	***	ns	ns	ns	*	ns
C14:0	ns	***	ns	ns	ns	*	ns
C15:0	***	ns	ns	ns	ns	ns	ns
C16:0	ns	*	ns	ns	ns	*	ns
C16:1	***	ns	ns	ns	ns	*	ns
C17:0	***	ns	ns	ns	ns	ns	ns
C18:0	***	ns	ns	ns	ns	ns	ns
C18:1 trans	***	ns	ns	ns	ns	ns	***
C18:1	***	ns	ns	ns	ns	***	ns
C18:1 n-7	***	***	*	*	ns	ns	***
C18:2 n-6	***	*	***	ns	ns	ns	ns
C18:3 n-3	***	**	**	ns	ns	ns	ns
WBC[1]	***	ns	ns	ns	ns	ns	ns
Total fatty acids	*	ns	ns	ns	ns	ns	*

ns = not significant; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$, WBC[1]-weight of branched chain fatty acids

Table 5.7a. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the loin adipose tissue of sheep*

	% by weight of total fatty acids												
	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
Fatty acid	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
C10:0	0.27	0.24	0.36	0.19	0.29	0.21	0.26	0.20	0.36	0.26	0.27	0.22	0.045
C12:0	0.16	0.14	0.23	0.10	0.14	0.12	0.17	0.11	0.18	0.10	0.19	0.09	0.033
C14:0	2.96	2.74	3.76	2.45	2.77	2.60	2.75	2.26	3.23	2.42	3.25	2.29	0.329
C15:0	1.88	1.70	1.70	2.00	1.52	1.28	1.39	1.52	1.06	1.04	1.00	0.82	0.239
C16:0	20.93	20.84	24.02	19.41	20.35	21.47	20.62	19.15	24.07	21.78	25.53	22.49	1.823
C16:1	2.72	3.03	2.90	3.07	2.25	2.49	2.41	2.48	2.60	2.75	2.43	2.49	0.165
C17:0	4.23	4.24	3.84	4.53	3.99	3.44	3.86	4.37	2.93	3.28	3.24	2.41	0.500
C18:0	9.38	9.29	10.34	7.77	10.46	9.91	10.62	10.51	12.39	12.07	14.12	12.48	1.322
C18:1 trans	1.50	1.22	1.92	1.15	1.54	1.51	2.19	1.51	4.44	2.67	3.28	5.45	0.673
C18:1 n-9	28.67	30.80	29.68	31.05	27.04	29.61	27.19	27.48	27.43	29.25	28.06	27.37	1.308
C18:1 n-7	0.85	1.04	0.87	1.09	0.69	0.85	0.89	0.76	0.89	0.90	0.80	1.04	0.075
C18:2 n-6	1.87	1.87	1.96	2.07	1.57	1.35	2.12	1.71	1.10	0.94	1.08	1.16	0.177
C18:3 n-3	1.71	1.54	1.79	1.58	4.43	3.40	5.30	4.26	2.05	1.71	2.10	2.10	0.456
WBC[1]	17.21	15.86	11.60	17.69	14.32	12.58	12.65	14.82	7.96	10.36	7.00	7.25	2.886
Remaining fatty acids	5.67	5.46	5.03	5.86	8.65	9.20	7.60	8.84	9.31	10.48	7.65	12.33	1.097
Total fatty acids	94.71	94.90	95.27	94.56	91.91	91.41	92.86	91.74	91.34	90.25	92.88	88.47	1.032

WBC[1]-weight of branched chain fatty acids

Table 5.7b. *Effect of dietary fat, vitamin E and breed on the fatty acid composition of the loin adipose tissue of sheep (significance of main effects and interactions)*

	% by weight of total fatty acids						
	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
Fatty acid	sign	sign	sign	sign	sign	sign	sign
C10:0	ns	***	ns	ns	ns	ns	ns
C12:0	ns	***	ns	ns	ns	ns	ns
C14:0	ns	***	ns	ns	ns	ns	ns
C15:0	***	ns	ns	ns	ns	ns	ns
C16:0	**	*	ns	ns	ns	ns	ns
C16:1	***	*	ns	ns	ns	ns	ns
C17:0	***	ns	ns	ns	ns	ns	ns
C18:0	***	ns	ns	ns	ns	ns	ns
C18:1 trans	***	ns	ns	ns	ns	ns	***
C18:1 *n*-9	**	*	ns	ns	ns	ns	ns
C18:1 *n*-7	***	***	ns	*	ns	ns	**
C18:2 *n*-6	***	ns	**	ns	ns	ns	ns
C18:3 *n*-3	***	*	*	ns	ns	ns	ns
WBC[1]	***	ns	ns	ns	ns	ns	ns
Total fatty acids	***	**	ns	*	ns	ns	ns

ns = not significant; *= p<0.05; **= p<0.01; ***= p<0.001. WBC[1]-weight of branched chain fatty acids

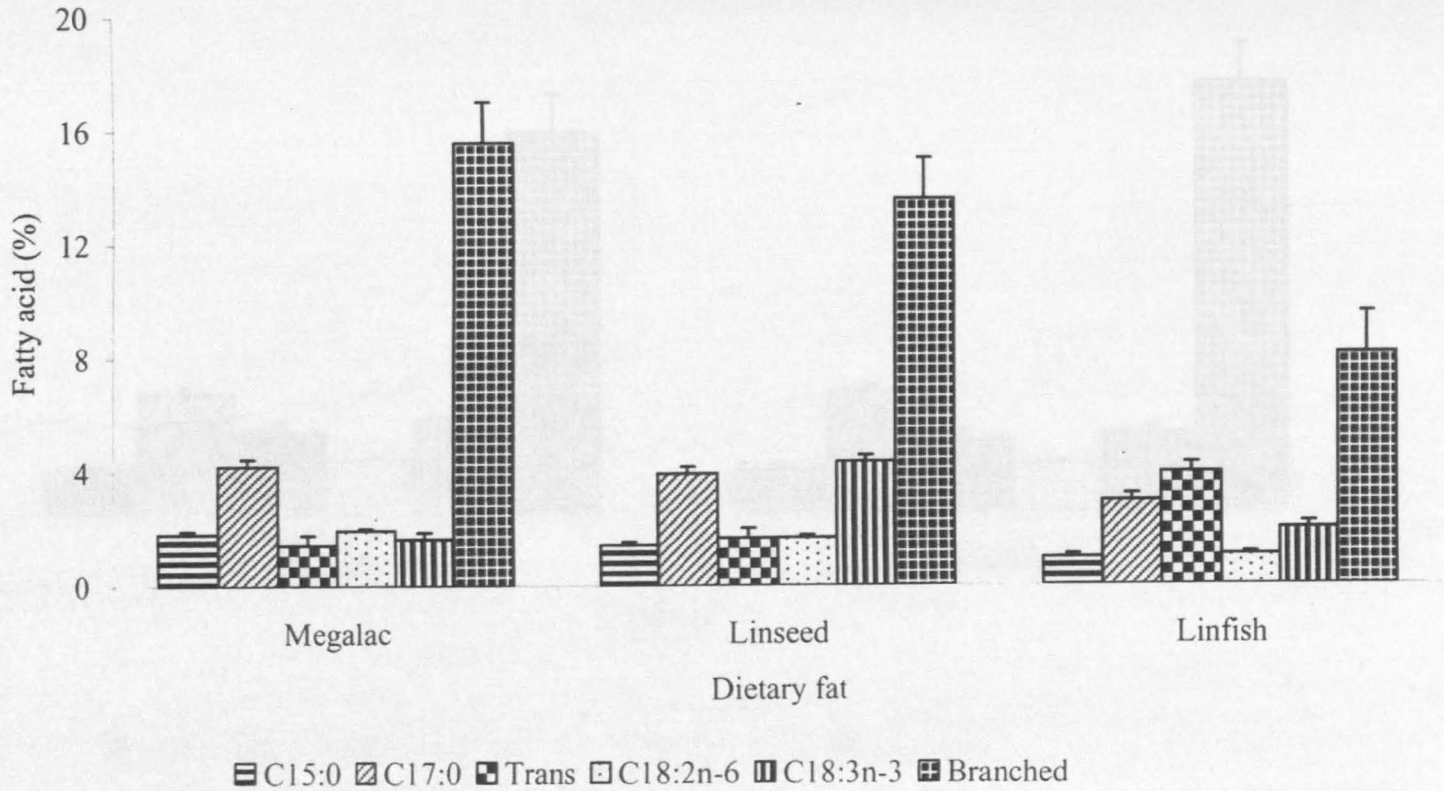


Figure 5.4 *Content of selected fatty acids in the loin adipose tissue of lambs fed diets with different fat sources*

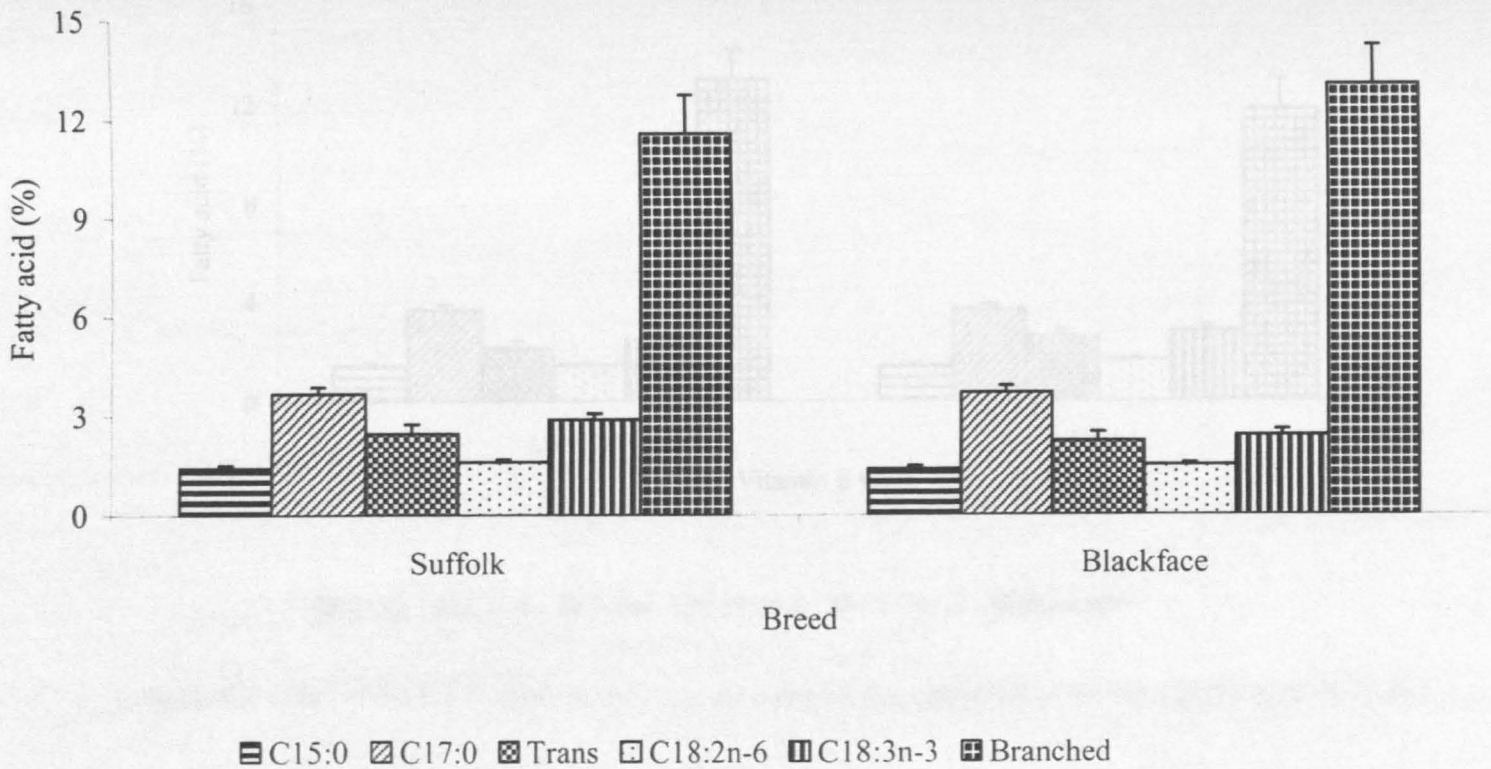


Figure 5.5 *Breed effects on the content of selected fatty acids in the loin adipose tissue of lambs*

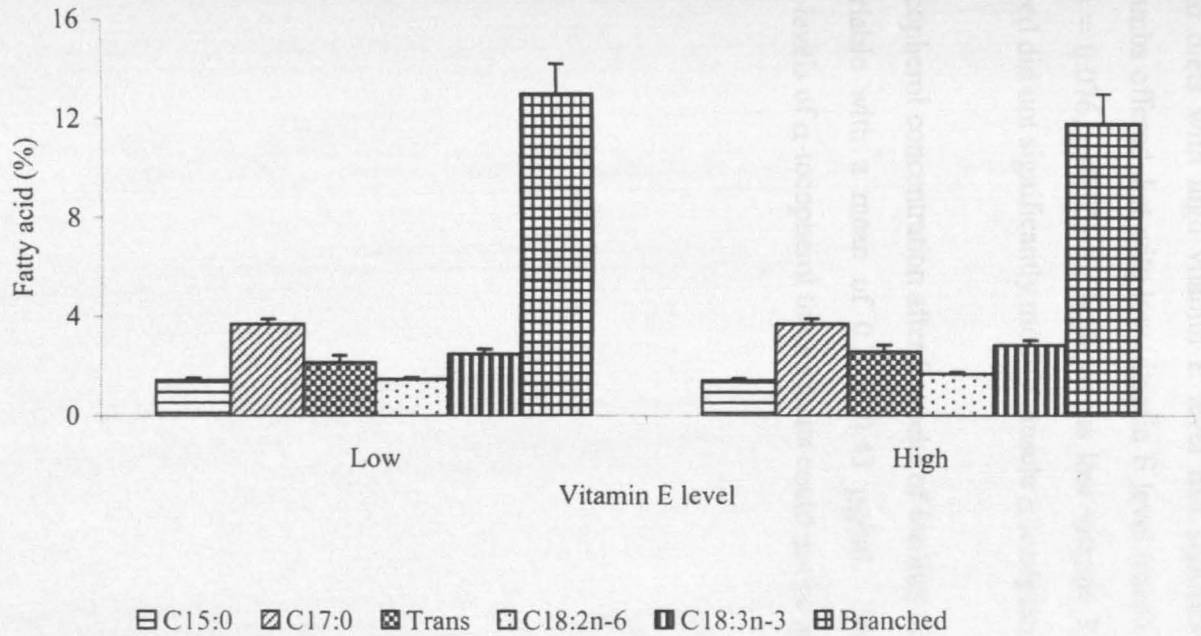


Figure 5.6 *Effect of vitamin E supplementation upon content of selected PUFA in the loin adipose tissue of lambs*

5.7 MUSCLE AND PLASMA ALPHA-TOCOPHEROL CONTENT

The levels of α-tocopherol in *longissimus dorsi* (LD) are presented quantitatively (mg/kg muscle) in Table 5.8a and the significance of the main effects and interaction are in Table 5.8b. Muscle vitamin E content was low and averaged 0.4 mg/ kg muscle. However, lambs offered diets with high vitamin E level had significantly higher tissue α-tocopherol levels than lambs offered diet with low vitamin E level (mean values were 0.52 *vs* 0.27 mg/kg muscle (sed = 0.076, $p<0.05$) for high versus low vitamin E diets, respectively. Dietary fat source or breed did not significantly modify muscle α-tocopherol content.

Plasma α-tocopherol concentration after 8 weeks of feeding the various dietary treatments was low and variable with a mean of 0.2 ± 0.43 μg/ml. Many plasma samples contained undetectable levels of α-tocopherol these results could not be analysed statistically.

Table 5.8a. *Effects of dietary fat, vitamin E and breed on the vitamin E content of longissmus muscle from sheep*

	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
(mg/kg muscle)	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
Vitamin E	0.20	0.18	0.48	0.51	0.55	0.23	0.51	0.29	0.27	0.17	0.37	0.95	0.186

Table 5.8b *Effects of dietary fat, vitamin E and breed on the vitamin E content of the longissmus muscle from sheep. (significance of main effects and interactions)*

	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
	sign	sign	sign	sign	sign	sign	sign
Vitamin E	ns	ns	*	ns	ns	ns	ns

ns = not significant; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$

5.8 DISCUSSION

5.8.1 Animal performance and carcass characteristics

The breeds examined in the current study were from two contrasting production systems in the UK sheep industry. The Suffolk (Downs breed), has undergone years of selection for increased growth rate, efficiency of feed utilisation and other qualities such as superior meat characteristics which makes it an ideal terminal commercial sire breed. In contrast, Scottish Blackface is a hill breed, naturally selected to survive harsh environmental conditions and seasonal variations in forage growth (Russell *et al.* 1968). Suffolk lambs ate more than the Scottish Blackface, but the difference was not apparent when expressed on a metabolic live weight basis but instead reflected differences in mature breed size or maintenance energy requirement. Likewise, Suffolk lambs had a numerically higher weight gain than the Scottish Blackface lambs (280 *versus* 240 g/day, respectively) although the difference was not apparent when expressed on a metabolic live weight basis. However, feed conversion ratio (FCR) was significantly lower in Scottish Blackface compared to Suffolk lambs. This maybe attributed to the heavier live weights (greater than half the mature live weight) at slaughter for Suffolk lambs, because as lambs mature the efficiency of feed utilisation decreases as the proportion of fat to lean deposition increases (Burton and Reid, 1969). In the previous study (Chapter 4 section 4.3.2), Suffolk lambs had a lower FCR (7.9 *versus* 4.7, for Suffolk lambs in the current *versus* previous study), when offered similar diets to those in the current study, but finished at a lower live weight (46.0 *versus* 43.0 kg, for Suffolk lambs in the current *versus* previous study).

Suffolk lambs had a superior carcass conformation and subcutaneous fat cover, than the Scottish Blackface lambs due to genetic differences in patterns of fat deposition. Russell and McClelland (1972) demonstrated that hill breeds contained less subcutaneous and more intermuscular fat than the Downs breed, which suggests that Hill breeds partitioned more energy towards accumulating energy reserves, to ensure survival during periods of limited feed resources. McClelland *et al.* (1976), concluded from a study of four sheep breeds that in general all breeds have a similar carcass composition (lean, bone and fat content) at the same degree of maturity. But Hammond (1932), had earlier pointed out that breeds such as the Suffolk, which have been selected for meat production, have considerably more fat (principally in the subcutaneous depot) relative to body weight than do primitive or semi-primitive breeds

such as the Soay and Shetland. These latter breeds have some similarities in conformation to the Scottish Blackface, neither of which has been selected for meat qualities and this may explain the observed differences in carcass characteristics in the current study.

Feeding lambs diets containing fish oil increased the level of subcutaneous fat cover, an observation similar to that in lambs fed diets containing fish oil in the previous growth trial. As previously discussed (Chapter 4; section 4.61 and Chapter 3; section 3.4.5) an increase in the production of propionate in the rumen of lambs offered diets containing fish oil may favour lipogenesis and consequently increased fat deposition (Garton *et al.* 1972 and Jones, 1982).

Suffolk lambs offered formaldehyde treated linseed had a poorer FCR than lambs offered whole linseed from the same breed in the previous trial. Mean FCR was 8.05 and 5.64, for Suffolk lambs offered diets containing formaldehyde treated linseed in the current and whole linseed in the previous study (Chapter 4; section 4.3.2), respectively. However, there was no significant effect of dietary fat on food intake, live weight gain or feed conversion ratio in the current study. In a number of studies, a reduction in voluntary food intake of both dry matter and live weight gain after including formaldehyde treated safflower in cattle diets at a level of up to 6 % oil has been reported (Faichney *et al.* 1972; Cuitin *et al.* 1975). Garrett *et al.* (1976) also observed reductions in feed consumption as a result of feeding formaldehyde treated soybean preparations. The rate of live weight gain was not reduced when cattle were offered formaldehyde supplements (6 % fat) and the improved feed conversion ratio was attributed to an increase in metabolisable energy supply derived from the protected lipid supplement (McCartor and Smith, 1978; Haaland *et al.* 1981).

In the current, study the effects of vitamin E supplementation level on animal performance ranged from positive effects to a lack of any significant effects. Copeland *et al.* (1995), looked at the growth and carcass characteristics of lambs fed diets containing 0, 100, 200 or 300 mg/kg vitamin E per day to finish (from 34.1 to 46.9 kg live weight) and reported no effects on food conversion, live weight gain or feed intake. However, in trials conducted with cattle moving from pasture to high-energy diets in confinement, gain and feed conversion efficiency were significantly improved by supplemental dietary vitamin E (Hill *et al.* 1990). This inconsistency may partly result from the variability in diet composition, vitamin E level, stress or previous animal history.

5.8.2 Muscle fatty acids

Most polyunsaturated fatty acids (PUFA) are found in the phospholipid fraction of the intramuscular lipids compared to the neutral lipid fraction, with the exception of the adipose tissue (Marmer *et al.* 1984). Alpha linolenic acid (C18:3*n*-3) is an essential fatty acids and the biochemical precursor for the *n*-3 fatty acid. As such most *n*-3 fatty acid in lamb muscle increased when diets containing formaldehyde treated whole linseed were fed to lambs. Generally, larger amounts of C18:3*n*-3 have been reported in the tissues of grass fed compared to concentrate fed ruminants (Marmer *et al.* 1984 and Enser *et al.* 1998), but the relative proportions are greater in the current study than in grass fed lambs. For example, the percentage intramuscular composition of C18:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 was on average 2.2, 1.0, 0.89 and 0.32 in grass fed lambs (Enser *et al.* 1998), compared to 3.7, 1.3, 1.2 and 0.28, respectively in this study when lambs were offered the linseed diet. This suggests that although feeding diets containing linseed almost doubles the concentration of C18:3*n*-3 in muscle lipids, proportional increases in longer chain *n*-3 C20 and C22 fatty acids is limited. Additionally, in the current study, marked improvements in C20:5*n*-3 and C22:5*n*-3 concentrations were not accompanied by increases in C22:6*n*-3 deposition. However, feeding preformed C20 and C22 fatty acids in the linfish diet increased the deposition of these acids in muscle. For example the concentration of C20:5*n*-3 was 1.1, 1.3 and 1.8 % in lambs fed the control, linseed and linfish diets, respectively in the current study.

The metabolic conversion of C18:3*n*-3 to C20:5*n*-3 parallels that of C18:2*n*-6 to C20:4*n*-6 derivatives (Brenner, 1989). Desaturation and chain elongation of both C18:3*n*-3 and C18:2*n*-6, to form C20 derivatives requires • 6 desaturase enzyme. Although •6 desaturase enzyme prefers C18:3*n*-3 over C18:2*n*-6, C18:3*n*-3 remains at a disadvantage due to several metabolic processes. First, compared to C18:3*n*-3, C18:2*n*-6 is preferentially deposited into structural membranes (Marmer *et al.* 1984) for further desaturation. Secondly, the oxidation of non-esterified fatty acids increases with increased number of double (Enser, 1984). This may suggest therefore that compared to C18:2*n*-6, C18:3*n*-3 is oxidised prior to esterification during absorption in the small intestines. Overall, animals posses a limited ability to convert C18:3*n*-3 to C20:5*n*-3 and C22:6*n*-3, whereas C18:2*n*-6 is desaturated and elongated to C20:4*n*-6 (Nettleton, 1991). In the current study, dietary C18:3*n*-3 to C18:2*n*-6 ratio was greater than 3.0 in the linseed diet. However the intramuscular content of C18:3*n*-3 and C18:2*n*-6 was 3.7 and 3.4 %, respectively, whilst that of C20:5*n*-3 and C20:4*n*-6 was 1.3 and 1.0 %, respectively for

lambs offered diets containing formaldehyde treated linseed. However, C18:2*n*-6 and C20:4*n*-6 fatty acids were displaced by offering lambs preformed C20 and C22 PUFA from fish oil in the linfish diet, to levels of 2.8 and 0.8 % respectively.

Previous studies have demonstrated that ruminant tissues can be markedly enriched with longer chain *n*-3 fatty acids. For example supplementing dairy cattle diets with fish oil, increased the plasma concentrations of C20:5*n*-3 from 0.7 (control) to 7.2 % of total fatty acids (Wonsil *et al.* 1994). Mandell *et al.* (1997) supplemented the diets of steers with fish meal and observed an increase in C20:5*n*-3 from 5.2 (control) to 29.8 mg/100 g muscle, although this level was substantially lower than 52.5 mg/100 g, observed in the current study in lambs fed the linfish diet. However, a maximum level of C22:6*n*-3, of 11 mg/100 g muscle was reported by Mandell *et al.* (1997) after feeding steers fish meal for 112 days, which is similar to the levels of 13.5 mg/100 g reported in the current study after feeding the linfish diet, compared to 19.2 and 26.47 mg/100 g muscle in the previous study (Chapter 4), in lambs offered the linfish and fish oil diets, respectively. This variability in deposition of preformed C20 and C22 fatty acids from fish oil, especially within experiments maybe attributed to differences in rumen biohydrogenation. Contrary to the work of Ashes *et al.* (1992a), C20 and C22 fatty acids from fish oil were biohydrogenated current work (Chapter 3, section 3.3.3) and in the work of Doreau and Chilliard, (1997).

Lambs offered diets containing formaldehyde treated linseed alone in the current study contained a similar level of C22:6*n*-3 to that in control lambs (7.8 mg/100 g). In contrast, offering lambs diets containing linseed in the previous study (Chapter 4), increased C22:6*n*-3 from 8.5 (control) to 13.0 mg/100 g muscle. This observation coupled with the higher concentrations of C18:0 in lambs offered diets containing formaldehyde treated linseed suggest extensive biohydrogenation of dietary C18:3*n*-3 in the rumen. Ashes *et al.* (1979) argued that the effectiveness of formaldehyde treated lipid supplements was largely determined by the technology used in the production process. The degree of protection should however be as high as 80 % *in vitro* and 60 % *in vivo*. *In vitro* studies of Gulati *et al.* (1997), have demonstrated the ability of formaldehyde treated fats, prepared from canola oilseed (rapeseed), to resist rumen hydrogenation. For example C18:2*n*-6 was hydrogenated by 14 %, which equates to 86 % rumen protection.

Rumen protection can also be assessed on the basis that animals are unable to synthesise essential fatty acids (Hood *et al.* 1976). Thus the concentration of these acids and their metabolites in muscle lipids would be an index for the degree of rumen protection. Using a formula modified from the work of Hood *et al.* (1976) and Ashes *et al.* (1979), the *in vivo* protection of the formaldehyde treated C18:3*n*-3 in the current study can be calculated as follows;

$$\text{Protection (\%)} = \left[\frac{100 \times (\text{Actual C18:3n-3} + \text{Metabolites in muscle lipids})\%}{(\text{Theoretical (control) C18:3n-3} + \text{Metabolites in muscle lipids})\%} \right]$$

Using the data for Suffolk lambs in Table 5a, formaldehyde treatment afforded C18:3*n*-3 in linseed a 15 % rumen protection compared to 12 % with whole linseed in the previous study (Chapter 4). This may partly explain the similarities in intramuscular concentrations of C8:3*n*-3 and its metabolic derivatives in lambs offered diets containing either whole linseed or formaldehyde treated linseed in the previous and current studies.

Scott *et al.* (1970), demonstrated that the level of C18:2*n*-6 could be raised to over 20 % of the total fatty acids in ruminants fat. This was achieved by feeding specific quantities of dietary oils high in C18:2*n*-6 protected from rumen biohydrogenation by micro-encapsulation in formaldehyde treated casein. The increases were accompanied by corresponding decreases in the proportion of C16:0, C18:0 and C18:1*n*-9 in the subcutaneous tissue (Scott *et al.* 1971). Similar changes have been observed in the intramuscular lipids of animals offered diets supplemented with protected rapeseed oil seeds or fish oil (Ashes *et al.* 1993). By contrast, St. John *et al.* (1987) and Ekeren *et al.* (1992) reported no change in the fatty acid composition of tissues from steers offered diets containing whole rapeseed or sunflower in an unprotected form. These contradictions can be attributed to differences in rumen biohydrogenation, length of feeding, age of animals and the degree of fatness.

Oleic (C18:1*n*-9) and palmitic (C16:0) fatty acids predominated in lambs offered the two diets containing formaldehyde treated linseed, when the data was presented quantitatively (mg/100 muscle), whereas the inverse was true when the data was presented as a proportion of the total fatty acids (%). For example, 100 g of muscle from lambs fed on the formaldehyde treated linseed and linfish diets contained 1136 and 1029 mg, respectively, of C18:1*n*-9 as opposed to

876 mg from an equal portion of muscle from control (Megalac) fed lambs. By contrast, the concentration of C18:1*n*-9 was 36.3 and 33.5 % of the total fatty acids in lambs fed the formaldehyde treated linseed and linfish diets, respectively compared to 37.5 % in control fed lambs. The apparent contradiction in the two formats arises from the relative leanness of muscles from lambs offered the control diet. Thus the infiltrated fat (neutral lipids) and its accompanying fatty acids, contained more C18:1*n*-9 in Megalac (control) fed lambs, but the fat was present in lesser amounts than in lambs offered diets containing formaldehyde treated linseed and linfish diets.

Dietary fish oil doubled the concentration of *trans*-11 C18:1 (vaccenic acid) in muscle lipids as previously reported in milk from cows fed diets containing fish oil (Wonsil *et al.* 1994; Mansbridge and Blake, 1997) or in lambs in the previous study (Chapter 4). Ruminally derived *trans* fatty acids result from incomplete microbial biohydrogenation of C18 unsaturated fatty acids (Harfoot and Hazlewood, 1988), because animals lack the enzymes for significant production of *trans* isomers *in situ* (Wahle and James, 1993). Epidemiological studies suggests that the various isomers found in commercially hydrogenated oils and in ruminant fats may differ from each other in their negative effects on metabolism and cell function. The predominant *trans*-11 C18:1 (vaccenic acid) isomer in ruminant fats is reported to be less detrimental to health than *trans*-9 C18:1 (elaidic acid) isomer which is more abundant in hydrogenated vegetable oils (Willet *et al.* 1993).

5.8.3 Adipose tissue fatty acids

Long chain C20 and C22 fatty acids were not detected in the subcutaneous adipose tissue of lambs offered any of the dietary treatments. This maybe attributed to the low proportion of phospholipid in adipose tissue as well as the failure of ruminants to incorporate these fatty acids into triacylglyceride fractions (Storry *et al.* 1974; Ashes *et al.* 1992; Enser *et al.* 1996). Ruminants also selectively incorporate both C18:2*n*-6 and C18:3*n*-3 into membrane lipids (Sanders, 1988), which is an adaptation to rumen biohydrogenation, which causes ruminants to be delicately balanced with respect to essential fatty acids.

The adipose tissue of lambs offered the formaldehyde treated linseed diet contained higher C18:3*n*-3 levels, than that reported in lamb at retail (4.3 *vs* 0.97 % of total fat respectively in the study of Enser *et al.* (1996). Although the amount of dietary C18:3*n*-3 escaping rumen

biohydrogenation, in the current is not known, the level of C18:3*n*-3 deposited in the subcutaneous adipose tissue was close to that reported in monogastric animals. In the study of Cunnane *et al.* (1990), growing pigs fed diets containing 5 % flax (2.3 % C18:3*n*-3), for 8 weeks contained 5.8 % C18:3*n*-3 in the adipose tissue compared to 4.3 % in the current study. Moore *et al.* (1969), reported that when sheep were given intra-abomasal infusions of linseed oil, maize oil or linoleic acid, C18:3*n*-3 and C18:2*n*-6 increased in the plasma triacylycerides 1.5 hours after infusion. However, in the plasma phospholipid, increases in C18:3*n*-3 and C18:2*n*-6 began 8-9 hours and 24-25 hours, respectively after infusion of the emulsions. This observation has been attributed to the rapid turnover of triacylglyceride-carrying lipoprotein fractions compared with that of other plasma lipid fractions (Lascelles *et al.* 1964). This implies that whilst C18:2*n*-6 is preferentially esterified into the phospholipid fraction, C18:3*n*-3 is incorporated into the triacylglycerol fraction. Almost all polyunsaturated fatty acids (PUFA) (especially essential fatty acids, C18:2*n*-6 and C18:3*n*-3) are preferentially esterified to cholesterol esters and phospholipid fractions (Noble, 1984). However, when the absorption of PUFA are in excess of that normally encountered (for example feeding protected fats), the phospholipid synthesis is insufficient to accommodate the PUFA supply and extensive incorporation into triacylglycerides becomes apparent (Cook *et al.* 1972) as in the current study. There is evidence to suggest that C18:3*n*-3, is deposited in the triacylglycerides fraction rather than in membrane lipids in ruminants (Moore *et al.* 1969, Marmer *et al.* 1984 and Enser *et al.* 1996). For example the C18:2*n*-6 to C18:3*n*-3 ratio is lower in the adipose tissue than muscle, 1.4 versus 1.9 in lamb, 2.3 versus 3.4 in beef, and in pigs 10 versus 14.7 (Enser *et al.* 1996). This may account for the higher content of C18:3*n*-3 in the adipose tissue (storage lipids) compared to intramuscular lipids in the current study.

Supplementing lamb diets with formaldehyde treated linseed, which is high in C18:3*n*-3 in the current study, decreased the concentration of C18:2*n*-6 in the adipose tissue. For instance, lambs fed the control diet contained the highest level of C18:2*n*-6 in the adipose tissue compared to lambs on the linseed and linfish diets. This suggests that the increased availability of C18:3*n*-3, displaced C18:2*n*-6 from the adipose tissue lipids. This competition is similar to that discussed previously with the muscle fatty acids (section 5.8.2). There is evidence to suggest that the competition between C18:3*n*-3 and C18:2*n*-6 differs between organs and results in a lower incorporation of C18:2*n*-6 into the adipose tissue and heart lipids or lower metabolism to more unsaturated products in the liver and kidney lipids compared to the brain

lipids (Cunnane *et al.* 1990).

Lambs fed diets supplemented with formaldehyde treated linseed also contained higher levels of C18:0 than lambs on the control diet. This may have resulted from the greater absorption of C18:0 in the small intestines as a result of to rumen biohydrogenation of dietary C18 fatty acids. Additionally, • 9 desaturase enzyme which converts C18:0 to C18:1*n*-9 in the adipose tissue maybe inhibited by the increased polyunsaturation in the adipose tissue (St. John *et al.* 1991). This hypothesis may be supported by the significant decreases in C18:1*n*-9 concentration and consequently increased C18:0 concentration, in lambs fed the linseed and linfish diets compared to control lambs. Although not reported in ruminants, increased polyunsaturation depresses Δ9 desaturase activity in mouse liver (Enser, 1979).

Lambs offered the linseed diet in the current study contained lower levels of C16:0, which is the principal end product of fatty acids synthesis *de novo*, compared to lambs on the control or linfish diets. Vernon, (1976) observed that fatty acid synthesis in lamb adipose tissue was inhibited by dietary tallow (high in C18:1*n*-9). However, Hood *et al.* (1980) observed inhibition of fatty acid synthesis in the adipose tissue of lambs *in vivo*, only when they were fed protected safflower oil, which is high in C18:2*n*-6, and not when the protected lipid was either beef tallow (2.5 %, C18:2*n*-6) and palm oil (9.1 %, C18:2*n*-6). Thus the high levels of mono- and polyunsaturated fatty acids in the adipose tissue of lambs offered the linseed diet in the current study may both have decreased *de novo* fatty acid synthesis.

The adipose tissue is the major lipogenic organ in ruminants (Vernon, 1981). As such, it contains fatty acids synthesised *de novo* by the animal, as well as acids not present in the diet or present in small undetectable amounts in the intramuscular lipids. This explains the presence of both odd (C17:0 and C15:0), and branched chain fatty acids in the adipose tissue and suggests that dietary components, in addition to fatty acids may have a significant effect on the fatty acid composition of the adipose tissue, partly by influencing the *de novo* synthesis of fat (Enser, 1991). Duncan *et al.* (1972), observed that when lambs were offered diets containing 90 % rolled barley, their adipose tissue contained a greater proportion of odd and branched chain fatty acids with a lower melting point (soft fats) than conventionally fed lambs. Garton *et al.* (1972), demonstrated that propionate was a precursor for the synthesis of odd chain fatty acids which was enhanced when fat was added to lamb diets (Gibney and L'estrange, 1975).

Similarly, the branched and odd chain fatty acids, in the current study, may have resulted from *de novo* fatty acid synthesis from increased propionate in the rumen, although volatile fatty acids were not quantified.

Branched and odd chain fatty acid, may also arise from *de novo* microbial fatty acid synthesis in the rumen from carbohydrate as well as amino acid precursors, and constitute 10-20 % of the total fatty acid in post ruminal digesta (Ferlay *et al.* 1993). However, in the metabolism experiment (Chapter 3, section 3.3.6), diets containing fish oil decreased microbial protein synthesis and consequently microbial fatty acids available for absorption and deposition into tissues. Dietary long chain PUFA are known to defaunate the rumen (Ikwuegbu and Sutton, 1982), but the effects of defaunation on the composition of rumen volatile fatty acids is variable. Many authors have reported that defaunation frequently results with an increase in the molar proportions of propionate at the expense of acetate and butyrate as discussed in experiment one (Chapter 3 section 3.4.5). Likewise long chain PUFA in the rumen, may also decrease the total volatile fatty acid concentration (Kurihara *et al.* 1978), and consequently decrease the substrate (propionate) available for the synthesis of odd or branched chain fatty acids. This may explain the observed low concentration of branched and odd chain fatty acids in the adipose tissue of lambs offered diets containing fish oil (linfish diet) in the current study.

Miller *et al.* (1981), observed that in range and feedlot steers, medium chain saturated acids (C10:0 to C15:0) were distributed throughout all 3 positions of intramuscular triacylglycerides, whilst C16:0 predominated in the *sn*-1 positions, C18:0 and C20:0 in the *sn*-1 and *sn*-3 positions. Branched chain fatty acids C13:0 and C15:0 predominated in the *sn*-2 position and were essentially absent from the *sn*-3 position while monounsaturated acids, with the exception of *trans* C18:1, were found mainly in the *sn*-2 and *sn*-3 positions. *Trans* C18:1 predominated in the *sn*-1 and *sn*-3 positions thereby resembling a long chain saturated acids. Polyunsaturated fatty acids C18:2*n*-6 and C18:3*n*-3 were equally divided between positions *sn*-2 and *sn*-3. Hence the low content of branched fatty acids in lambs fed the linfish in the current study maybe attributed to the competition for incorporation from the C18:1*n*-9 which constituted the major fatty acid in the adipose tissue. Likewise the high *trans* C18:1 concentration may be due to the ability of *trans* unsaturates to esterify onto the *sn*-1 position, thus avoiding competition with C18:1*n*-9. However, it is difficult to ascertain in this study, the extent to wish the triacylglyceride structure determined the fatty acid composition of the adipose tissue, but lambs

offered the linfish diet contained the highest level of *trans* C18:1, a by-product of incomplete biohydrogenation and a reflection of the extent to which fish oil can limit rumen metabolism.

5.8.4 Vitamin E effects

The ARC (1980) and NRC (1984) estimates of vitamin E requirement in ruminants are 22.4 and 15-60 mg/kg DM, respectively. The criteria used for determining these dietary requirements are largely based on the prevention of deficiency status in the animal. Putman and Comben (1987) recommended that dietary vitamin E should be based on the level of dietary polyunsaturated fat based on observations in calves, that polyunsaturated fatty acids (PUFA), especially C18:3*n*-3, escaping rumen biohydrogenation was associated with a rise in plasma creatine kinase, indicating muscular degenerative myopathy (Rice *et al.* 1981). Other studies found that providing colostrum rich in vitamin E, enhanced disease resistance and immune response in the neonate and this could be achieved by feeding high levels (60 mg/kg DM) of dietary vitamin E to ewes before lambing (Njeru *et al.* 1994). Dietary vitamin E levels ranging from 400 to 2000 mg/kg DM, elevate muscle vitamin E concentrations, and consequently reduce lipid peroxidation, post slaughter in beef (Arnold *et al.* 1993), pork (Monahan *et al.* 1992), poultry (Lin *et al.* 1989) and lamb (Wulf *et al.* 1995). Arnold *et al.* (1992) suggested that the critical factor in using vitamin E to extend shelf life of beef, was implementing a supplementation regimen that achieved a minimum muscle tissue vitamin E concentration of approximately 3.0 to 3.5 mg/kg meat (fresh) (Faustman *et al.* 1989). Lamb containing α-tocopherol in excess of 3.0 mg/kg, did not appear to have any benefit in terms of reducing lipid peroxidation post slaughter (Wulf *et al.* 1995).

Although in excess of current recommendation, dietary vitamin E levels of 100 and 500 mg/kg DM in the current study were not effective in enhancing plasma or muscle in lambs fed any of the dietary treatments. Optimal levels of plasma α-tocopherol are usually considered to be at least 0.5 μg/ml, based on concentrations associated with protection against *in vitro* erythrocyte haemolysis, in mammalian species. However, levels of 4.07 μg/ml have been reported in ewes lamb after 28 days supplementation with 60 IU per day of dl-α-tocopherol acetate (Njeru *et al.* 1994). In the current study plasma vitamin E was less than 0.2 μg/ml after feeding lambs diets containing 100 or 500 mg/kg DM for 60 days, whist muscle vitamin E was less than 3 mg/kg tissue. The causes of deficiency are incompletely understood but may be due to a number of factors.

5.8.4.1 Vitamin E metabolism in the rumen

The disappearance of vitamin E from the rumen increased from 8 to 42 %, as the cornstarch content in the diet increased from 20 to 80 % in sheep diets (Alderson *et al.* 1971). Orally administered vitamin E did not appear in the venous blood (Alderson *et al.* 1971). The authors implicated ruminal microbes in the disappearance of dietary vitamin E from the rumen. Indirectly supporting this hypothesis, Rode *et al.* (1990), quantified vitamin A disappearance in rumen fluid from cattle fed concentrate, hay or straw diets. The authors estimated the effective degradation of vitamin A as 67, 16 and 19 %, respectively. In contrast to vitamin A, the double bonds of tocopherols are within an aromatic ring, which are not readily saturated or degraded anaerobically under prevailing rumen conditions and retention times (Chesson and Forsberg, 1988). Leedle *et al.* (1993) reported that vitamin E was not microbially degraded, during 24 hours *in vitro* incubation in ruminal contents (pH 4.9-5.7) from concentrate fed animals. This contradicts the preintestinal vitamin E losses observed by Alderson *et al.* (1971) while feeding a high cornstarch diet. The discrepancies in the latter study have been associated with inadequate extraction or prediction of vitamin E transport, or vitamin E oxidation during collection of digesta due to lack of adequate protection (Rouquet *et al.* 1992; Leedle *et al.* 1993).

The rumen environment has indeed been accredited with increasing availability of vitamin E (supplemented as α-tocopherol acetate), suggesting that any treatment resulting in a bypass of ruminal fermentation, could lower post ruminal availability (Hidiroglou *et al.* 1990; Hidiroglou *et al.* 1992). Alpha tocopherol quinol also acts as a reductant during biohydrogenation in the rumen (Hughes *et al.* 1982). It is possible that after hydrolysis of the ester bond in α-tocopherol acetate, α-tocopherol was reduced to the quinone form whilst acting as a reductant during biohydrogenation. The quinone form of α-tocopherol has no antioxidant capacity and is excreted in the urine (Gallo-Torres, 1980).

5.8.4.2 Tissue metabolism

The absorption of vitamin E is low and only 20-40 % is absorbed in the small intestines (Bender, 1992). This absorption is enhanced by medium chain triacylglycerides and inhibited by polyunsaturated fatty acids (PUFA) (Gallo-Torres, 1980). The latter effect has been attributed to the chemical interactions between tocopherol and PUFA or their peroxidation products in the intestinal lumen. PUFA also occupy relatively more space in the lipoproteins

and as such displace tocopherol or inhibit its binding capacity and consequent absorption.

In a series of studies in rats, Green *et al.* (1967) demonstrated that when PUFA were offered together with α-tocopherol, only half of the α-tocopherol was recovered from the rats given C18:2*n*-6, compared to rats offered C18:1*n*-9. However, when the PUFA were given separately from the α-tocopherol these differences were not apparent. Feeding cod liver oil instead of lard decreased tissue α-tocopherol concentration and accelerated vitamin E deficiency symptoms in rats, but this difference was not apparent when the interaction between the cod liver oil and α-tocopherol in the digestive tract was avoided. The addition of a synthetic antioxidant to the diet eliminated the negative effect of the PUFA supplements on α-tocopherol absorption (Green *et al.* 1967). Glyceride enhanced the absorption of α-tocopherol, while C18:2*n*-6 decreased it to 7 % (Gallo-Torres, 1973). The authors attributed this effect to a destruction of α-tocopherol in the gut by PUFA. Higher tissue and plasma levels of α-tocopherol when radio labelled free tocopherol was dosed into the duodenum than when dosed into the rumen (Hidiroglou and Jenkins (1974); Hidiroglou *et al.* (1970). Akerib and Steiner (1971), demonstrated in rats that in the absence of bile salts, vitamin E absorption was practically inhibited. However, when bile salts were introduced into the system, vitamin E absorption was significantly impaired by the addition of unsaturated lipids (only 55 % of the vitamin E was absorbed in the presence of the unsaturated lipid compared to 81 % in the presence of glucose alone).

Whilst it is possible that PUFA destroyed some vitamin E and consequently impaired absorption, in the current study there were no differences in plasma vitamin E concentrations despite differences in dietary PUFA composition. However, although the control fat consisted of predominantly a saturated fatty acids the concentration of C18:2*n*-6 was similar across the diets and ranged between 12 to 16 % of the total dietary fatty acids. This acid is preferentially protected from rumen biohydrogenation by a preferential and proportional incorporation into free fatty acid vacuoles of solid adherent bacteria (Bauchart *et al.* 1989). Compared to the control fat, C18:3*n*-3 in linseed and fish oil fatty acids have a negative effect on microbial efficiency in the rumen as reported in Chapter 3, section 3.3.6. Although the crude protein content of all the diets was high (17 %), microbial protein synthesis would be higher in lambs offered the control diet and consequently a higher amount of C18:2*n*-6 escaping rumen biohydrogenation or interacting with α-tocopherol prior to absorption. PUFA especially

C18:2*n*-6 are preferentially incorporated into structural membranes (Marmer *et al.* 1984) and act as potential peroxidants thus compromising the antioxidant level. Although muscle PUFA levels were higher in lambs offered the linseed and linfish diets, reflecting the higher duodenal flow of dietary PUFA, lambs offered the linfish diet contained significantly higher muscle vitamin E. This may be attributed to the additional vitamin E protection afforded by the synthetic antioxidant (500 ppm BHT) added to fish oil during storage which protects vitamin E.

In the current study, lambs offered the high vitamin E diets contained higher levels of nutritionally desirable PUFA, (C18:3*n*-3, C20:5*n*-3, C22:6*n*-3 and C18:2*n*-6) than lambs on the low vitamin E diets, which contained lower levels of muscle vitamin E. Similarly, birds offered diets with antioxidant contained higher levels of C18:3*n*-3, total *n*-3 PUFA plus lower levels of C16:0 than birds offered diets without antioxidant (Ajuyah *et al.* 1993). However, despite the low vitamin E status (both plasma and muscle) which can technically be interpreted as deficiency in the current study, lambs did not express vitamin E deficiency symptoms. It could well be that other defence mechanism operating *in vivo* prevented or minimised tissue damage thus preventing clinical deficiency symptoms. One such defence mechanisms involve selenium as part of the glutathione peroxidase enzyme, which operate in the subcellular membranes. The level of glutathione peroxidase in tissues depends on the concentration of selenium in the diet (Rice and Kennedy, 1982). In current study, selenium levels in the diet were high and averaged 1.0 mg/kg DM compared to the recommended levels of 0.3 mg/kg DM (ARC, 1980). Although not quantified this high selenium level may have increased the level of glutathione peroxidase enzyme in tissues.

5.9 CONCLUSIONS

Dietary fat source and vitamin E level did not affect growth performance. Suffolk lambs had a significantly higher food intake and food conversion ratio than the Scottish Blackface lambs. Compared to the control fat, formaldehyde treated whole linseed increased total fat content and the level of C18:3*n*-3 and C20:5*n*-3, whilst fish oil increased fat content and the level of C20:5*n*-3 and C22:6*n*-3 in muscle. The level of C18:3*n*-3 in the subcutaneous adipose tissue after feeding diets containing protected linseed were close to the maximum for retention of high meat quality, based on results from pigs. Vitamin E was poorly absorbed and deposited into muscle tissue of lambs on any of the dietary treatments, but lambs offered high vitamin E diets contained significantly higher muscle vitamin E and polyunsaturated fatty acids than lambs on the low vitamin E diets.

CHAPTER 6

NUTRITIONAL IMPLICATIONS

6.1 INTRODUCTION

Dietary fat plays a role in the aetiology of coronary heart disease (CHD) in several ways. First, the development of atherosclerotic plaques involves the accumulation of lipids, particularly cholesteryl esters, which are derived from plasma lipoproteins (Department of Health, 1994). Secondly, lipids are also involved because of their role in eicosanoids synthesis and in the formation of thrombi, which block arteries and cause CHD (Gurr and Harwood, 1996). The COMA report on the nutritional aspects of cardiovascular disease (Department of Health, 994) recommended that fat should not provide more than 35 % of the dietary energy intake, that saturated fatty acids should not exceed 10 % of dietary energy intake, and that *trans* unsaturates should not exceed 2 % of dietary energy and should be reduced. No change in the proportion of PUFA in the human diet was required, since the polyunsaturated to saturated fatty acid (P:S) ratio currently approaches the value of 0.45 recommended in the previous report (Department of Health and Social Security, 1984). Additionally, the COMA report advised that the intake of long chain *n*-3 PUFA should be increased to a value of 0.2 g/day and that the ratio of *n*-6 to *n*-3 fatty acids should be reduced to below 2.0. Based on these guidelines, four factors are thought to be important when judging the nutritional value of fat in foods. These are the total fat content, *trans* fatty acids, saturated fatty acids, P:S ratio and the *n*-6:*n*-3 ratio.

6.2 RESULTS AND DISCUSSION

6.2.1 Total fat content

Total fat content and fatty acid ratios related to healthy nutrition in the *semimembranosus* muscle of lamb for the first growth trial (Chapter 4) and the second growth trial (Chapter 5) are presented in Tables 6.1 and 6.2a & b, respectively. Foods below 5 g of fat/100 g are generally considered to be low fat (Food Advisory Committee, 1990). On this basis muscle tissue trimmed of any visible fat (less than 5 % fat), as in the current studies would qualify for inclusion in a healthy diet. Mean values for total fat were 2.6 and 2.8 % for lambs in the first growth trial (Table 6.1) and second growth trial (Table 6.2) respectively. Enser *et al.* (1996) observed that the fat content of meat cuts could increase to values of over 20 % of tissue weight when visible subcutaneous and intermuscular fat were included.

When considering the quantitative relevance of the fat content of meat to human nutrition, 100 g is used to represent an average serving of meat. This is the serving size used in the Meat and Livestock Commission (MLC) recommendations, and similar quantities were used by COMA (Department of Health 1994). The fat contained in 100 g of lean lamb from the current study would provide approximately 1.0 % of the daily energy intake for men (2450 kcal) and 1.4 % for women (1640 kcal). Enser *et al.* (1996) reported a higher daily energy intake from lamb chops at retail (1.8 and 2.6 %, respectively for men and women). Additionally, the authors observed that when a 100 g serving consisted of muscle and adipose tissue (assuming 20 % adipose tissue overall), fat contributed 9.1 % of the average daily energy intake for men and 13.3 % for women. While both options can clearly be included as part of a complete diet and still meet the COMA nutritional targets, the selection of leaner portions (removal of visible fat) would contribute towards a lower fat diet and compensate for consumption of meat products which may be high in fat overall, especially products where fat is not easily removed, such as sausages, pies and pasties. Nute *et al.* (1983) reported that approximately half the population removed visible fat from meat.

Table 6.1 *Effect of dietary fat and breed on fatty acid content and fatty acids ratios in semimembranosus muscle in sheep*

	mg/100 g muscle															
	Megalac			Linseed			Fish oil			Linfish				Diet	Breed	[5]D x B
Fatty acid	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	Suffolk	Soay	Friesland	SED	sign	sign	sign
Total fat	2728	2406	2681	2700	2190	2391	3267	2298	2797	2657	2834	2241	459.5	ns	ns	ns
Trans C18:1	97.2	90.7	103.5	167.8	113.1	166.7	266.1	160.0	173.6	226.6	235.2	153.5	42.89	***	ns	ns
Σ SFA[1]	1144	969	1101	1027	818	835	1276	871	1088	1003	1064	832	196.1	ns	ns	ns
Σ MUFA[2]	1035	824	1020	916	712	807	964	639	889	823	853	718	157.6	ns	ns	ns
Σ PUFA[3]	272.1	346.9	256.0	305.2	327.9	318.4	391.6	354.8	366.8	303.4	339.5	286.5	29.26	***	*	ns
Σ n-6 PUFA	183.9	233.5	171.9	143.0	167.5	160.6	135.6	136.0	138.1	129.0	152.7	131.1	14.54	***	***	*
Σ n-3 PUFA	88.2	113.3	84.1	162.2	160.4	157.8	256.0	218.8	228.7	174.4	186.8	155.4	22.36	***	ns	ns
Ratios																
P:S[4]	0.23	0.33	0.24	0.26	0.35	0.34	0.28	0.35	0.32	0.25	0.26	0.32	0.042	ns	**	ns
Σ n-6/Σ n-3	2.09	2.10	2.24	0.88	1.07	1.12	0.53	0.64	0.65	0.74	0.86	0.88	0.203	***	ns	ns
C18:2/C18:3	3.36	3.58	4.15	1.29	1.50	1.74	2.59	2.45	3.25	1.61	2.06	2.16	0.410	***	**	ns

[1]SFA= Saturated fatty acid; [2]MUFA= Monounsaturated fatty acids; [3]PUFA= polyunsaturated fatty acids

[4]P:S = Ratio of polyunsaturated to saturated fatty acids; [5]D x B = Diet x Breed interaction

Table 6.2a. *Effect of dietary fat, vitamin E and breed on the fatty acid content and fatty acid ratios in the semimembranosus muscle of sheep*

	mg per 100 g muscle												
	Megalac				Linseed				LinFish				
	Low		High		Low		High		Low		High		
	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	Suffolk	Blackface	SED
Total fatty acids	2517	2345	2660	1806	3060	2804	3101	3429	3162	2952	3241	2767	424.8
Trans C18:1	46.9	35.8	57.9	23.8	62.9	48.0	60.3	61.7	109.4	81.1	95.9	118.0	16.55
Σ SFA[1]	1036	937	1089	689	1250	1150	1258	1414	1341	1281	1425	1226	187.5
Σ MUFA[2]	1057	999	1091	734	1215	1156	1255	1386	1223	1131	1249	1042	193.7
Σ PUFA[3]	218.5	207.9	243.3	212.4	296.7	239.3	332.1	317.0	256.5	232.3	277.3	246.9	25.82
Σ n-6 PUFA[3]	134.1	134.0	148.8	138.2	132.8	112.3	163.5	135.5	117.4	98.6	123.8	103.4	11.94
Σ n-3 PUFA[3]	109.2	99.9	120.4	102.3	201.4	162.8	206.2	227.5	185.3	188.8	203.0	207.8	20.15
[5]P:S	0.22	0.23	0.23	0.31	0.24	0.22	0.27	0.24	0.21	0.19	0.20	0.21	0.024
Σ n-6/Σ n-3	1.26	1.34	1.26	1.36	0.69	0.70	0.83	0.62	0.65	0.54	0.62	0.50	0.071
C18:2/C18:3	1.88	2.25	1.83	2.28	0.88	0.96	1.02	0.88	1.19	1.24	1.19	1.21	0.128

[1]SFA= Saturated fatty acid; [2]MUFA= Monounsaturated fatty acids; [3]PUFA= polyunsaturated fatty acids;[5]P:S = Ratio of polyunsaturated to saturated fatty acids

Table 6.2b. *Effect of dietary fat, vitamin E and breed on the fatty acid content and fatty acid ratios in the semimembranosus muscle of sheep (significance of main effects and interactions)*

	mg per 100 g muscle						
	Fat	Breed	Vitamin E	Fat x Breed	Fat x Vitamin E	Breed x Vitamin E	Fat x Breed x Vitamin E
	sign	sign	sign	sign	sign	sign	sign
Total fatty acids	***	ns	ns	ns	ns	ns	ns
Trans C18:1	***	ns	ns	ns	ns	ns	ns
Σ SFA[1]	***	ns	ns	ns	ns	ns	ns
Σ MUFA[2]	*	ns	ns	ns	ns	ns	ns
Σ PUFA[3]	***	**	**	ns	ns	ns	ns
Σ n-6 PUFA[3]	***	***	**	ns	ns	ns	ns
Σ n-3 PUFA[3]	***	ns	*	ns	ns	ns	ns
[5]P:S	***	ns	*	*	ns	ns	ns
Σ n-6/Σ n-3 ratio	***	ns	ns	**	ns	ns	ns
C18:2/C18:3	***	**	ns	**	ns	ns	ns

ns = not significant; *= p<0.05; **= p<0.01; ***= p<0.001

6.2.2 Trans fatty acid

The quantity of *trans* isomers in 100 g of muscle from both growth studies was low, although higher values were recorded in lambs offered the linfish diet. In the first growth trial (Table 6.1) the concentration of *trans* C18:1 were 96.8, 148.9, 197.1 and 204.8 mg/100 g muscle (sed = 23.95, $p<0.001$) for lambs offered the Megalac, linseed, fish oil and linfish diets, respectively (Table 6.1), and in the second growth trial (Chapter 5) mean values were 41.1, 58.1 and 101.1 mg/100 g muscle (sed = 8.28, $p<0.001$), for lambs offered the Megalac, linseed and linfish diets, respectively (Table 6.2). Using the highest value of 204.8 mg/100 g muscle, the *trans* fatty acids in 100 g serving portion of lamb from the current study would supply 0.08 % of the average daily energy intake for men and 0.11 % for women. Both values are below the maximum recommended level of 2 % (Department of Health 1994).

Isomeric fatty acids, particularly *trans* isomers have been implicated in the aetiology of various metabolic and functional disorders such as increasing erythrocyte fragility, mitochondrial swelling and enhancing the arrhythmogenecity of cardiomycocytes in animal preparations possibly by modulating cell membrane physico-chemical characteristics (British Nutrition Foundation, 1987). Incorporation of isomeric fatty acids into tissue phospholipids is potentially more important than the incorporation into other lipid classes since changes in their fatty acid composition will modify membrane structure and function. When these isomers are incorporated into the *sn*-2 position of the membrane phospholipids they alter the essential fatty acid (EFA) precursor availability and consequently eicosanoid synthesis (British Nutrition Foundation, 1987). However, *trans* fatty acids, particularly *trans* monounsaturates, are similar to saturated fatty acids in being preferentially incorporated into the *sn*-1 position of the phospholipids and the *sn*-1 and *sn*-2 positions of the triacylglycerols. This is in marked contrast to the *cis* isomer C18:1*n*-9, which is randomly distributed between the various positions in these lipids (Wahle and James, 1993). Varying degrees of selectivity towards both *cis* and *trans* isomers in man and animals have been reported during the synthesis of phospholipids (Beare-Rogers, 1988). *Cis*-12 C18:1, an isomer of C18:1*n*-9 which is also formed during hydrogenation., is selectively incorporated into the *sn*-2 position of phospholipids, which makes it a more likely candidate for impairing eicosanoid synthesis than *trans* isomers. Recent epidemiological studies suggest that vaccenic acid the major *trans* isomers in ruminant fats, which has a double bond in the C11 position as, opposed to

elaidic acid, the main isomer in partially hydrogenated vegetable oil, which has a double bond in the C9 position, is not a risk factor for CHD (Willet *et al.* 1993).

The most important effects of isomeric fatty acids appear to relate to their competitive interaction with EFA metabolism. Many of the reported detrimental effects of *trans* isomers in animals are purported to result from EFA deficiency, rather than a specific effect of *trans* isomers *per se*, since they can be apparently prevented by increasing EFA availability (Gurr, 1993). This is thought to be due to inhibition of EFA metabolism, specifically at the level of the Δ6 and Δ5 desaturase enzymes responsible for converting C18:2*n*-6 to C20:4*n*-6 as evidenced by the increase in C18:2*n*-6 and the decrease in C20:4*n*-6 they elicit in tissue phospholipids (Wahle and James, 1993). This suggestion is supported by observation that trans isomers inhibit desaturase enzyme activity in human cell lines in culture (Wahle *et al.* 1991).

6.2.3 Saturated fatty acids

In the first growth trial (Table 6.1), the intramuscular concentration of total saturated fatty acids (SFA) (mean value 998 mg/100 g muscle) and was not affected by dietary fat, breed or the interaction of the two factors. However, in the second growth trial the (Table 6.2), the intramuscular concentration of SFA was higher in lambs offered diets containing formaldehyde treated linseed. The mean values for SFA were 938, 1268 and 1318 mg/100 g muscle for lambs fed the Megalac, linseed and linfish diets, respectively, (sed = 93.8, $p< 0.001$). This was mainly due to differences in fat content (Table 6.2), which was higher in lambs offered the linseed or linfish diets compared to control lambs, and reflects their greater carcass fatness.

The saturated fatty acids in meat vary in chain length, the most frequently occurring being palmitic (C16:0), stearic (C18:0), myristic (C14:0) and lauric (C12:0) acids. Evidence suggests that their cholesterol raising property result from the saturation and suppression of low-density lipoprotein (LDL) receptors (Keys *et al.* 1965);

$$\Delta C = 1.35(2\Delta S - \Delta P) + 1.52$$

Where ΔC = LDL cholesterol; ΔS = saturated fat and ΔP = polyunsaturated fat (PUFA)

This equation indicates that the incremental effect of saturated fatty acids on plasma cholesterol is twice as effective at raising plasma cholesterol as the hypocholesterolaemic effect of polyunsaturated fatty acids. While C14:0 and C16:0 are most effective at raising plasma cholesterol, C18:0 and fatty acids with 10 or less carbon atoms appear to have no cholesterol raising effects (Bonanome and Grundy, 1988). Because C16:0 contributes substantially to the total amount of saturated fatty acids in animal fat (over 20 % by weight of total fatty acids), a decrease in C16:0 and an increase in C18:0 or C18:*n*-9 is a favourable change with regard to current nutritional guidelines. In the current work, feeding lambs diets containing whole linseed decreased C16:0 and increased C18:0. In the first growth study, mean values for C16:0 were 24.4, 20.7, 24.1 and 22.9 % (sed = 0.40, $p<0.001$) and mean values for C18:0 were 13.5, 13.3, 11.3 and 11.4 % (sed = 0.43, $p<0.001$) for lambs offered the Megalac, linseed, fish oil and linfish diet, respectively. Therefore dietary inclusion of linseed provides the potential to improve the nutritional quality of lamb, by reducing its plasma and LDL cholesterol raising properties.

6.2.4 Polyunsaturated to saturated fatty acid (P:S) ratio

6.2.4.1 Effects of dietary fat

In the first growth trial lambs offered the fish oil diet contained higher PUFA levels than control lambs. The mean values were 292, 317, 370 and 310 mg/100 g muscle (sed = 16.34, $p<0.001$, Table 6.1) for lambs offered the Megalac, linseed, fish oil and linfish diets, respectively. However, in the second growth trial PUFA levels were highest in lambs offered the linseed diet. The mean values were 220.5, 296.2 and 253.2 mg/100 g muscle (sed = 12.91, $p<0.001$, Table 6.2) for lambs on the Megalac, linseed and linfish diets, respectively. In both trials, the P:S ratios were low according to the Department of Health (1994) guidelines, which recommends a value of 0.45 for the diet as a whole. In the first growth trial (Table 6.1) supplementing lamb diets with *n*-3 PUFA sources increased the P:S ratio from 0.28 in control lambs to 0.33, whilst in the second growth trial the inclusion of formaldehyde treated linseed resulted in a P:S ratio of 0.29 compared to 0.26 in control lambs (Table 6.2). Thus although PUFA uptake was increased, significant biohydrogenation of dietary PUFAs also occurred leading to even greater uptake and deposition of saturated fatty acids. But despite this low P:S ratio, there is clearly considerable scope for the consumption of additional fat high in PUFA to

give an overall P:S ratio of 0.45 in the human diet. Furthermore by selecting leaner cuts as in the current study, the scope for consumption of other fats in the diet within the recommended guidelines is greatly increased.

Earlier work in Australia using formaldehyde treated whole sunflower seeds reported increased intramuscular concentrations of C18:2*n*-6 of 14 % by weight of total fatty acid in lamb (Ackerson and Johnson, 1975), and consequently a higher P:S ratio than that observed in the current study. In non-ruminants providing whole linseed in the diet can increase the P:S ratio. For example, broilers offered diets containing (250 g/kg) whole linseed for 3 weeks contained 14 % C18:3*n*-3 in the thigh muscle (Scaife and Wachira, 1998). This illustrates the potential extent to which lamb muscle maybe amenable to manipulation.

6.2.4.2 Breeds effects

In the first growth trial (Table 6.1), Soay lambs had a higher intramuscular concentration of PUFA than the Friesland but not the Suffolk lambs. The mean values were 316, 342 and 306 mg/100 g muscle (sed = 14.10, $p<0.05$, Table 6.1) for Suffolk, Soay and Friesland lambs, respectively. The P:S ratio was higher in Soay than in Suffolk lambs. In the second growth trial, Suffolk lambs contained a higher intramuscular concentration of PUFA than the Scottish Blackface lambs. The mean values were 271 and 243 mg/100g muscle (sed = 10.54, $p<0.01$, Table 6.2) for Suffolk and Scottish Blackface lambs, respectively. Although the P:S ratio was similar between the two breeds, there was a significant interaction between diet and breed with Suffolk lambs having a higher P:S ratio than Scottish Blackface lambs when offered the linseed diet. The mean values were 0.31 and 0.27 (sed = 0.021, $p<0.01$) for Suffolk and Scottish Blackface lambs, respectively. When considering the quantitative relevance of the PUFA content in the different breeds, it can be argued that the advantage of Soay lambs over Friesland, or Suffolk over Scottish Blackface lambs, can readily be offset in the human diet by varying the intake of other significant and readily available sources of PUFA or saturated fatty acids, since it is the P:S ratio in the diet as a whole which is significant. Overall, the beneficially higher P:S ratio in Soay and Suffolk lambs in the first and second growth trials respectively was due to the higher proportion of *n*-6 fatty acids in the total PUFA, because the total *n*-3 PUFA did not vary significantly between breeds. Whereas, PUFA especially those of

the *n*-6 series, tend to lower LDL cholesterol level, fatty acids from the *n*-3 series have not been shown to have consistent effects on blood cholesterol (Wiseman, 1997). However, the *n*-3 long chain fatty acids from fish oil are more effective in reducing triacylglycerol levels which may independently contribute to CHD risk.

6.2.5 *n*-6 to *n*-3 ratio

6.2.5.1 Effects of dietary fat

Linoleic acid (C18:2*n*-6) and α-linolenic acid (C18:3*n*-3) are essential fatty acids, because mammals lack the ability to introduce the *n*-6 and *n*-3 double bond (Brenner, 1989). Evidence suggests that although the elongation and desaturation of these acids does occur in man, the process is inefficient and the conversion of C18:2*n*-6 to C20:4*n*-6 (arachidonic acid) and C18:3*n*-3 to C20:5*n*-3 (eicosapentaenoic acid), compete for the Δ6 desaturase enzyme (Nettleton, 1991). Consequently, a diet rich in C18: 2*n*-6 but poor in C18:3*n*-3 generates a relative excess of C20:4*n*-6, and a relative deficiency of C20:5*n*-3 and C22:6*n*-3 (docosahexaenoic acid) (Sanders *et al.* 1978). In the current work lambs offered the linseed diet (whole or formaldehyde treated linseed) had a high levels of C18:3*n*-3 in the intramuscular lipids. The mean values were 85.6 and 113.4 mg/100 g muscle for lambs in the first and second growth study, respectively. The supply of C18:3*n*-3 to the human diet would offset the competition between C18:2*n*-6 and C18:3*n*-3 for Δ6 desaturase enzyme and consequently lead to the desaturation and elongation of C18:3*n*-3 to longer chain fatty acids (James *et al.* 1992). These longer chain derivatives are incorporated into membrane phospholipids where the C20 PUFA serve as precursors for biologically active compounds known as eicosanoids (Gurr and Harwood, 1996). The relative excesses of C20:4*n*-6 leads to the production of eicosanoids, involved in a range of stress related disorders including cardiovascular diseases and inflammatory conditions which can be depressed by eicosanoids derived from C20:5*n*-3 (Sargent, 1997). For example thromboxane A_3 from C20:5*n*-3 is significantly less pro-aggregatory than thromboxane A_2 from C20:4*n*-6 (Sinclair and O'Dea, 1990). Theoretically, remodelling the phospholipid composition through dietary manipulation would alter the type of eicosanoids produced (Scott and Ashes, 1993). But overall, other factors, which regulate fatty acid incorporation and release into and from the phospholipid, will dictate the substrates available for eicosanoids synthesis.

In the first growth study, the *n*-6 to *n*-3 ratio (calculated as sum total *n*-6 fatty acids divided by sum total of *n*-3 fatty acids) was beneficially low in lambs fed diets with added *n*-3 PUFA sources. The mean ratios were 2.15, 1.02, 0.61 and 0.83 (sed = 0.113, $p<0.001$) for lambs on the Megalac, linseed, fish oil and linfish diet, respectively (Table 6.1). Likewise the C18:2*n*-6 to C18:3*n*-3 ratio was beneficially lower in lambs offered diets containing linseed compared to lambs offered the Megalac or fish oil diets. The mean ratios were 3.70, 1.51, 2.77 and 1.94 (sed = 0.228, $p<0.001$) for lambs offered the Megalac, linseed, fish oil and linfish diet, respectively (Table 6.1). In the second growth trial the *n*-6 to *n*-3 and C18:2*n*-6 to C18:3*n*-3 ratios were beneficially lower in lambs offered diets containing *n*-3 PUFA sources compared to control lambs, but all ratios were within the ideal value of 2.0 (Table 6.2). Other than elongation and desaturation of precursor acids, longer chain fatty acids such as C20:4*n*-6, C20:5*n*-3 and C22:6*n*-3 may be derived more efficiently from dietary sources. In the current work, the sum of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3 from 100 g of muscle from lambs offered the control, linseed, fish oil and linfish diets, would supply approximately 55, 81, 194 and 121 mg/person/day which is about 0.28, 0.41, 0.97 and 0.60, respectively of the daily recommended intake (0.2 g/day) by the Department of Health (1994). Although information on the specific role of C22:5*n*-3 is limited, metabolic pathways exist for its conversion to C22:6*n*-3 or C20:5*n*-3 (Voss *et al.* 1991) and this justifies its inclusion as part of the long chain *n*-3 PUFA in the current study. Besides fish and marine algae, meat lipids supply 19 % of the total intake of *n*-3 PUFA in the human diet, whereas 14 % comes from fish (Gregory *et al.* 1990), although levels of C20 and C22 *n*-3 PUFA in meat are less than in fish (Nettleton, 1991). Lamb from the current work would enhance C20 and C22 *n*-3 PUFA supply from meat to the human diet and reduce the incorporation of *n*-6 PUFA into membrane lipids.

One aim of raising the *n*-3 PUFA in the diet is to decrease the thrombotic tendency of blood and lower the risks of CHD. Arachidonic acid is the major thrombotic fatty acid and meat is considered to be an important source of this fatty acid (Kinsella *et al.* 1990), despite its synthesis by vegans (Sanders *et al.* 1978). However, meat is also the source of other antithrombogenic fatty acids C20:3*n*-6 and C20:5*n*-3. If the sum of these fatty acids divided by C20:4*n*-6 content is an indicator of the antithrombotic potential, values from 100 g of lamb in the current work are approximately 1.3 (for lambs offered the linseed diets) and 3.0 (for lambs offered the fish oil and linfish diets) in both studies (Table 4.4 and Table 5.4). This value is

higher than the value of 0.52 reported in the plasma phospholipids of subjects consuming a normal diet in England (Sinclair *et al.* 1994). Meat from lambs offered the linseed diet in both growth studies would not only be an effective means of increasing the supply of long chain *n*-3 PUFA in man, but would also lower the dietary intake of C18:2*n*-6 and C20:4*n*-6, the major thrombogenic fatty acid, which significantly decreased in lambs offered the linseed, fish oil and linfish diets in both growth studies. Although, the higher levels of C18:3*n*-3 in muscle and adipose tissue of lambs offered diets containing linseed would contribute to the dietary supply of *n*-3 PUFA, Sanders and Younger (1981), observed that the effects of administering different *n*-3 PUFA to human subjects was not equivalent and dietary C20:5*n*-3 and C22:6*n*-3 were more effective then C18:3*n*-3, as a means of increasing the proportion of these acids in tissue lipids.

6.2.5.2 Breed effects

In the first growth trial *n*-6 fatty acids were higher in the Soay than in either the Suffolk or Friesland lambs. The mean values were 147.8, 172.7 and 150.7 mg/100 g muscle for Suffolk, Soay and Friesland lambs, respectively. In the second growth trial Suffolk lambs contained the highest level of *n*-6 fatty acids with mean values of 136.7 and 120.3 mg/100 g (sed = 4.87, $p<0.001$) for Suffolk and Scottish Blackface lambs, respectively. However, there were no significant differences between breeds in muscle *n*-6 to *n*-3 ratio in the current work. In the first growth trial the C18:2*n*-6 to C18:3*n*-3 ratio was lower in the Suffolk and Soay lambs compared to Friesland lambs. The mean values were 2.21, 2.39 and 2.82 (sed = 0.020, $p<0.01$) for Suffolk, Soay and Friesland lambs, respectively (Table 6.1). In the second growth trial there was a significant interaction between diet and breed with Suffolk lambs having a higher *n*-6 to *n*-3 ratio than Scottish Blackface lambs when offered the linfish diet. The mean values were 0.64 and 0.52 (sed = 0.051, $p<0.01$, Table 6.2) for Suffolk and Scottish Blackface, respectively), whilst the C18:2*n*-6 to C18:3*n*-3 ratio was beneficially lower in Suffolk compared to Scottish Blackface lambs, although both ratios were within the ideal value of 2.0 (Table 6.2).

The supply of preformed C20 and C22 *n*-3 fatty acids to the human diet is an important advantage, which can offset the low P:S ratio in the current study. Although, comprehensive

studies on the nutritional benefits of meat with different fatty acid composition are lacking, Sinclair *et al.* (1994) showed that lean beef with high *n*-3 PUFA concentrations can beneficially raise plasma *n*-3 fatty acids in humans when given for several weeks. Further research is however required to determine the optimal levels of C20 and C22 *n*-3 PUFA in the human diet, since the *n*-6 to *n*-3 ratio does not account for differences in fatty acid chain length which dictate *in vivo* metabolism and incorporation into membrane phospholipids.

6.3 ECONOMIC IMPLICATION

There are few estimates for the efficiency of conversion of long chain *n*-3 fatty acids from feed into meat for ruminants. Although little information exists on fish oil supplementation in lamb diets, it is worthy looking at the economic effects of various inclusion rates, taking an all concentrate indoor finishing system which has some similarities to the system used in the current study. Generally, the production system is characterised by store lambs housed in the autumn and winter periods and fed a compound feed designed to produce growth rates averaging 150 g per day, a feed conversion ratio of 6:1, and a carcass weight of 18 kg. Assuming that fish oil costs £450 per tonne, figures for increased feed costs for various combinations of feeding period and inclusion rate of fish oil can be calculated and are presented in Table 6.3.

Table 6.3. *Total cost (p) and cost per kg carcass weight (p) of fish oil inclusion in lamb diets*

	Feeding period			
	30 days		60 days	
Inclusion rate (g/kg)	Total	Per kg carcass	Total	Per kg carcass
10	12.2	0.68	24.3	1.35
20	24.3	1.35	48.6	2.70
30	36.5	2.03	72.9	4.05

Data from *Sources of n-3 PUFA additional to fish oil in livestock diets* (MAFF project OC9514, 1997)

However, the opportunity to manipulate *n*-3 fatty acid sources through specific diet supplementation in the UK maybe limited because the vast majority of lambs are currently finished off grass. The one exception, in addition to the system described above, is the early

lamb system designed to produce high quality products for Easter market. This already attracts a premium mark-up of some 70 % compared with the main season lamb prices, so an additional premium of more than 5 p/kg for enhanced C20:5*n*-3 and C22:6*n*-3 products is unlikely (MAFF, 1997). Nevertheless, it may be that young lambs as in the current study may utilise dietary rumen protected fatty acids more efficiently than older lambs (store lambs) as they do for energy and protein (FCE = 3.0:1).

Ruminants cannot tolerate a diet with a high fat content especially PUFA, because of its effects on rumen metabolism, unless it is offered as a rumen protected lipid. The diets of grazing ruminants contain 30-40 g/kg of lipid, with C18:3*n*-3 as the predominant fatty acid. Ashes *et al.* (1992b) fed high levels of protected fish oil, equivalent to 80 g/kg of fat, and supplying 14 g C20:5*n*-3 and 9 g C22:6*n*-3 per kg diet to 35 kg wether sheep for 35 days. This level substantially increased the levels of C20:5*n*-3 and C22:6*n*-3 in the serum lipids and in muscle phospholipids, but not in either the subcutaneous adipose tissue or muscle triacylglycerols. The authors concluded that because the phospholipid fraction represents only a small proportion of the fat in ruminant meat, it does not appear to offer a means of providing humans with a significant source of these fatty acids. However, because the basic knowledge on possible inclusion rates and efficiencies of utilisation of fatty acid from other novel sources, for example marine algae, in ruminant diets is lacking, it is difficult to draw a more precise conclusion on the potential use of *n*-3 fat sources.

Mansbridge and Blake, (1997) observed that C20:5*n*-3 and C22:6*n*-3 were increased (3.92 *vs* 0.14 and 2.3 *vs* 0 g/kg total fatty acids, respectively) in milk following fish oil supplementation. Recent studies by Chilliard and Doreau, (1997) compared the effects of either a ruminal or duodenal infusion of fish oil (menhaden oil, 300 ml for 4 weeks). Milk yield was unaltered by the fish oil supplements but protein concentration was significantly reduced by the rumen infusion and milk fat concentration by both treatments. The addition of fish oil substantially increased the secretion of *trans*-C18:1 but only the duodenal infusion led to any substantial increase in C20:5*n*-3 and C22:6*n*-3 in milk. These results strongly suggest that fish oils supplements should be protected from rumen biohydrogenation.

In non-ruminants, the most effective approach for enhancing C20:5*n*-3 and C22:6*n*-3 in muscle has been supplementing fish oil in diets and only limited success has been reported with diets rich in C18:3*n*-3. This is illustrated by the results of Irie and Sakimoto (1992; Table 6.4) which showed increased concentrations of C20:5*n*-3 and C22:6*n*-3 after pigs were supplemented for four weeks with refined sardine oil at 60 g/kg.

Table 6.4 *Effect of supplementing (60 g/kg) the diet of pigs with refined sardine oil on fatty acid composition of body lipids*

	Backfat		Perirenal fat	
Fatty acid (g/kg)	Control	+ fish oil	Control	+ fish oil
C18:2*n*-6	108	98	79	75
C18:3*n*-3	19	22	14	21
C20:5*n*-3	1.0	12	1.0	15
C22:6*n*-3	4.0	13	6.0	19

Data from Irie and Sakimoto, (1992)

Similar responses to dietary fish oil have been noted in poultry meat (Huang and Miller, 1993). The observation of a preferential deposition of *n*-3 fatty acids into muscle tissue and in breast meat in particular, provides an economic benefit over ruminants because customers are willing to pay premium rates for choice carcass portion. Van Elsywyk (1996) confirmed that supplementing laying hens with menhaden fish oil can bring about substantial increases in C20:5*n*-3 and in particular C22:6*n*-3 in the egg yolk. There is already a substantial premium to be obtained from the market for enhanced egg quality. For example, free range eggs and perchery eggs already command a premium of 29 p and 20 p per dozen respectively over cage eggs and 8 p per dozen premium is being offered for 'four grain' (special nutrition of hens) eggs. If a premia of this magnitude can be obtained then it may be more economical to include fish oil and other novel *n*-3 PUFA sources in layer diets instead of ruminant diets.

It is clear that manipulation of the diet of farm animals can enhance the concentrations of C20:5*n*-3 and C22:6*n*-3 in meat and eggs and although further work needs to be done, this maybe an important means of enhancing the national diet with PUFA, particularly if consumption of oily fish does not increase substantially. As non-ruminants, both the avian

and the pig can respond dramatically in their tissue lipids to changes in dietary fat. The success of poultry meat is the result of a range of features that embrace both a healthy profile with regard to total fat content and quality. By contrast the manipulation of unsaturated fatty acids of ruminant products continue to provide a challenging problem. In spite of considerable research over a period of 40 years, the inherent biohydrogenative capacities within the rumen have so far resisted attempts to readily allow manipulations. The extent to which specialist diets (for example formaldehyde protected lipid supplements) have been introduced commercially appears to be minimal, for, inspite of their undoubted success metabolically they have found little favour in terms of several major features such as economics, convenience and safety. However, as opposed to the contribution of health and well being through the provision of conventional PUFA, the biohydrogenation processes of the rumen may themselves provide an answer. Thus there are substantial recent data (Belury, 1995 and Jiang *et al.* 1996) denoting the anti-carcinogenic properties of conjugated *cis-trans* dienoic acid (*cis*-9-*trans*-*11*-C18:2), that is a prominent intermediary of rumen biohydrogenation. It seems ironic, therefore, that a feature of rumen metabolism that has been the focus of such enthusiastic attention over some 50 years in order to achieve its suppression, should now find itself centre stage with a view to promotion within ruminant meats and dairy products.

It is also clear from the current study that differences in aspects of tissue lipid composition and metabolism exist between the rare and commercial breeds when offered the control diet. Attention to such differences may not be wholly academic but posses practical implications of which advantage could be taken for both the intensively reared animal and the consumer alike. Clearly there exists quite an extensive ability to manipulate the unsaturated fat composition of ruminant animals and their products. However, economic and market situations as presently exist may not allow for any alternative. If animal products are to maintain their accepted eminent role as part of our daily diet, there must be willingness not only to face up to the problems, but to have the means to overcome them.

6.4 CONCLUSION

Dietary factors were more effective than genetic factors in enhancing the *n*-3 PUFA content of sheepmeat in the current work. However, further research is required on other sources of *n*-3 PUFA in ruminant feeds as well as more efficient and economical methods of protecting PUFA from rumen biohydrogenation.

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